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Pretreatment Methods for Enhancing Conversion of Lignocellulosic Material to Liquid Fuel

by

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ABSTRACT

A 2-litre steam gun has been assembled and instrumented. Measured steam consumption roughly equalled heat lost during loading and cook, plus that theoretically required. For 250 g wood with 250 g moisture, theoretical was 53 g to heat wood, 132 g to heat moisture, and 23 g to pressurize preheated gun to 240°C. Measured temperature rise, inside simulated wood chips, indicated cold under-cooked centres. Equal rates of pentosan solubilization and equal rates of destructions were found for thin wood whether wet or dry. Acid impregnation permitted lower temperatures and more pentosan solubilization for the same pentosan destruction with destruction of only a few percent of the cellulose. Rates of accumulation of pseudolignin and furfural indicate independant processes. Pyrolysis appears to be a major process. Oxygen content of wood decreased, with elimination of CO_2 and H_2O.

Inhibitors of both enzymatic hydrolysis and of fermentation are removed from steam-exploded wood by water extraction. Principal inhibitors were shown not to be: xylose, xylan, furfural, hydroxymethylfurfural or original wood extractives; but to be volatile (in part), and extractable by ethyl acetate but not by ether.

CHAPTER 1

TABLE OF CONTENTS

	Page
INTRODUCTION	7
DESCRIPTION OF STEAM GUN AND INSTRUMENTATION	8
STEAM CONSUMPTION	11
STEAM-EXPLODED WOOD (SEW) - SIX SERIES	16
SERIES 1	16
SERIES 2	16
SERIES 3	17
Recovery From Gun	17
Moisture Content of SEW-Implications For Steam Consumption	17
Yield and Pentosan Content of Water-Soluble Fraction	21
Water Insoluble Fraction	21
SERIES 4	25
SERIES 5	28
SERIES 6	30
CORRELATION OF DATA FROM FORINTEK AND IOTECH GUNS	32
TEMPERATURE RISE INSIDE WOOD SPECIMENS	34
PROCESS OF PENTOSAN DESTRUCTION	40

LIST OF TABLES

TABLE 1. (Series 1, Closed Cyclone, 2-L Gun, 240°C) Recovery From Gun, and Destruction of Pentosan 45

TABLE 2. (Series 2, Closed Cyclone, 2-L Gun, 240°C), Repeat of Series 1) Recovery From Gun 46

TABLE 3. (Series 3, Open Cyclone, 2-L Gun, 240°C) Recovery From Gun 47

TABLE 4. (Series 3 continued, Open Cyclone, 2-L Gun, 240°C) Amount, and Pentosan Content of Water-Solubles Fraction of Steam-Exploded Wood 48

TABLE 5. (Series 3 continued, Open Cyclone, 2-L Gun, 240°C) Pentosan, Lignin and Glucan Contents of Water-Washed Steam-Exploded Wood (WSEW), (i.e. of the Water-Insoluble Fraction)[2] 49

TABLE 6. (Series 4, Open Cyclone, 200-mL Gun, Dry Wood, 240°C) Recovery of Steam-Exploded Wood from Gun, and Amount and Analysis of Water-Soluble Fraction, when Wood Loaded into Gun was Air-Dry instead of Wet 50

TABLE 7. (Series 4, Open Cyclone, 200-mL Gun, Dry Wood, 240°C) Pentosan and Lignin Contents of Water-Washed Steam-Exploded Wood (WSEW), i.e. of the Water-Insoluble Fraction (WI) 51

TABLE 8. (Series 5, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 220°C) Recovery from Gun, and Water Extraction 52

TABLE 9. (Series 5 continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 220°C) Analysis of Whole Steam-Exploded Wood, Before Extraction With Water 53

TABLE 10. (Series 5 continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 220°C) Analysis of Water Solubles (WS) of Water Extract 54

TABLE 11. (Series 5 continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 220°C) Analysis of Water-Washed Steam-Exploded Wood (WSEW) (i.e. the Water-Insoluble Fraction of Steam-Exploded Wood) 55

TABLE 12. (Series 6, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 240°C) Recovery From Gun 56

TABLE 13. (Series 6 continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 240°C) Analysis of Whole Steam-Exploded Wood, Before Extraction With Water 57

TABLE 14. (Series 6 continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 240°C) Yield of Water Solubles in Water Extract, and Analysis of Water-Washed Steam-Exploded Wood (WSEW) (i.e. Water Insolubles) 58

TABLE 15. Composition of Permanent Gas in Steam Gun after 1 Minute at 240°C 59

LIST OF FIGURES

Fig. 1	Two-litre steam gun, before addition of insulation	60
Fig. 2	Comparison of theoretical weight of saturated steam required to heat 250 g of equivalent dry weight of aspenwood from 25°C to steam temperature, with that required to heat only the moisture content of the wood, for moisture contents from 10 to 125 percent (oven-dry basis)	61
Fig. 3	Comparison of theoretical weight of saturated steam required to fill void volume of two-litre gun charged with 250 g of equivalent dry weight of aspenwood of moisture contents 10 and 125 percent (oven-dry basis)	62
Fig. 4	Comparison of total theoretical weight of saturated steam required to heat 250 g of equivalent dry weight of aspenwood and moisture contents of 10 or 125 percent (oven-dry basis) from 25°C to steam temperature, and to fill void volume	63
Fig. 5	Comparison of theoretical and observed steam requirements for filling empty two-litre gun, with and without opening of lid, and the additional requirements for heating green aspenwood of equivalent dry weight 100 g and moisture content 51.97 percent (oven-dry basis)	64
Fig. 6	Recovery of steam-exploded wood from two-litre gun, using closed cyclone	65
Fig. 7	Recovery of steam-exploded wood from two-litre gun using open cyclone	66
Fig. 8	Comparison of amount of water-soluble pentosan in steam-exploded wood at 240°C, from originally wet or dry aspenwood, or at 220°C from 0.2 percent sulfuric-acid-impregnated aspenwood	67
Fig. 9	Comparison of amount of water-insoluble pentosan remaining in water-washed steam-exploded wood (WSEW) from originally wet or dry aspenwood, (pentosan contents from air-dry wood adjusted by multiplication by ratio of pentosan analyses of original woods)	68

Fig. 10 Pentosan content of steam-exploded aspenwood, before and after water extraction, showing more complete extraction of pentosan from acid-impregnated wood, for similar over-all destruction of pentosan 69

Fig. 11 Comparison of time in Iotech gun at 3.86 MPa (nominally 250°C) to corresponding time in two-litre gun at 240°C for same amount of pentosan destruction 70

Fig. 12 Temperature rise 3.2 or 6.4 mm beneath surface of green wooden specimens of different shapes and sizes, during heating in steam gun at 240°C 71

Fig. 13 Decrease in natural logarithm of temperature difference between steam temperature and wood temperature, 3.2 or 6.4 mm beneath surface of dowel-shaped specimens 72

Fig. 14 Decrease in natural logarithm of temperature difference between steam temperature and wood temperature, 6.4 mm beneath surface of rectangular blocks, in fibre direction and across fibre direction 73

Fig. 15 Destruction of pentosan during steam treatment of aspenwood at 240°C, and accompanying formation of pseudolignin and furfural, showing constant rate of formation of pseudolignin, independant of increasing furfural content 74

INTRODUCTION

A major goal of the work undertaken, under this contract, was an examination of the steam-explosion process itself and in particular a determination of mass and energy balances. Although it was originally intended that the small 200-mL Forintek steam gun would be used, the determination of meaningful heat and steam requirements in equipment with such a large surface/volume ratio appeared difficult. Because the construction of a larger 2-litre gun was already planned, it was decided to delay work on the process itself until the new gun would be available. The design of this larger gun was accordingly modified to facilitate this work and additional instrumentation was provided.

DESCRIPTION OF STEAM GUN AND INSTRUMENTATION

Figure 1 shows the steam gun before insulation was added. The vertically-mounted gun barrel is constructed of 316 stainless steel. It has an inside diameter of 63 mm, a wall thickness of 9.5 mm, and a volume of 2 litres. The upper end is fitted with a Parr bomb lid carrying a pressure gauge, a bleed valve and thermocouple probes which extend down into the gun. One of these can be adjusted, vertically to give the temperature at any point within the charge, along the axis of the gun barrel. The lower end of the barrel is connected to a reduced bore Kamyr ball valve (RNC 03-AAS) of 316 stainless steel with stellite seat rings. This ball valve is fitted with a double acting cyl. actuator, BC12, and a solenoid valve ASCO 8342B1. The barrel, lid and ball valve can be used at pressures of up to 4.14 MPa and temperatures of up to 254^{o}C.

Steam, produced in a high pressure boiler, capable of providing saturated steam at 250^{o}C, is introduced into the gun through either of two inlet pipes, one of which is located immediately below the lid, and the other immediately above the ball valve. When the ball valve is opened, the charge of steam and lignocellulosic material is violently discharged downward through this valve. After passing through a curved pipe of inside diameter 63 mm, also of 316 stainless steel, it enters the cyclone horizontally and tangentially.

The cyclone, also constructed of 316 stainless steel, has a diameter of 38 cm, and a height of 38 cm from its top to the upper end

of the conical bottom. The conical bottom has an angle of 60^{o} and empties through a 38 mm manually-operated ball valve. In order to facilitate quantitative recovery of the exploded product, the lid of the cyclone is removable. In normal operation, a light-weight lid of 316 stainless steel is used, with a 28-cm length of pipe (of diameter 20 cm), passing down through it vertically, to vent the steam axially from the centre of the cyclone. In order to condense and recover all of the volatile material produced in the gun, including all of the steam used, a second much heavier lid is available. It is a flat counter-balanced circular plate of 316 stainless steel, 25.4 mm thick, and is fitted with a 0.52 MPa rupture disc. This lid, shown in Figure 1, is bolted through a gasket to the flange at the top of the cyclone, and provides an air-tight seal. The total volume of gun, curved pipe and cyclone is 55 litres.

Thermocouple probes used to monitor the temperature were of type T (copper-constantan) with grounded measuring junctions sealed in a stainless steel 316 jacket. Both thermocouples conformed to the ISA and ANSI limit of error. The thermocouple probe, measuring the temperature within the charge of aspenwood chips in the gun, was 6.4 mm in diameter. The typical response time for this type of thermocouple is reported by the manufacturer to be 2.0 seconds (i.e. the time to reach 63.2 percent of the temperature change, as determined by ASTM STP 470A). The probe measuring the steam temperature was 3.2 mm in diameter with a reduced tip diameter of 1.6 mm. Its typical response time is reported to be 0.2 second.

Both thermocouples were connected to a Doric 402A digital temperature indicator (DTI), equipped with a five-thermocouple switching unit and an analog output module. The DTI provided digital linearization and cold-junction compensation for copper-constantan thermocouples. According to the manufacturer's data, its resolution and repeatability were $\pm$ 1°C, and the calibration drift was no more than $\pm$ 1° per year. The analog output of the DTI was recorded using a Hewlett-Packard 7100B dual-channel strip-chart recorder. In use, both thermocouples gave the same readings of the steam temperature.

An additional thermocouple probe was mounted in good contact with the outside surface of the gun barrel, under the insulation, at a point mid-way between the lid and the ball valve.

STEAM CONSUMPTION

The theoretical weights of saturated steam, at temperatures from 200 to 260°C, required to heat from 25°C to steam temperature a charge of 250 g of oven-dry aspenwood (associated with moisture contents of 0 to 125 percent, oven-dry basis) were calculated. The results are shown in Fig. 2, 3 and 4. It was assumed, as recommended by Stamm (1964), that heat of adsorption is negligible, and that moist wood can be considered as a simple mixture of dry wood and water. Stamm's relationship (Stamm, 1964), for the variation of the heat capacity of dry wood with temperature, was used, although a considerable extrapolation beyond Stamm's experimental data was required. The steam required for dry wood was relatively low, in any case, rising from 38 g to 64 g for temperatures from 200 to 260°C, as shown in Fig. 2. Also shown in Fig. 2 are a series of theoretical curves, for wood of different moisture contents, showing the steam requirements for heating only the moisture component present with the wood, from 25°C to steam temperature. Fig. 2 shows that, except for relatively dry wood, more steam is required to heat the moisture present in the wood, than is required to heat the dry wood itself. Freshly cut green aspenwood is commonly half wood, half moisture, i.e. moisture content approximately 100 percent. Fig. 2 shows that in this case, approximately two-thirds of the steam required to heat the green wood would be consumed in heating the moisture component.

Fig. 3 shows the relatively small weight of steam required to fill and pressurize the void volume in the gun, taking into account the volume of the wood and the condensate. Fig. 4 shows the total theoretical weight of steam required, i.e. the sum of the weights required to heat the wood, to heat the moisture, and to pressurize the void volume.

The results of initial experimental measurements of steam consumption are shown in Fig. 5. The lowermost curve of Fig. 5 shows the results obtained by pressurizing the otherwise empty gun with 240^{o}C steam, holding it for varying times with the inlet valve of the gun open to the boiler, and then firing the steam and accumulated condensate into the closed cyclone. Although the gun had been preheated to constant maximum temperature by the 240^{o}C steam before each of these experiments, the weight of condensate obtained even at zero time (i.e. by firing the gun immediately after pressurization without holding the gun under pressure) was approximately double the calculated theoretical amount. Furthermore, the positive slope of the curve shows an almost constant rate of production of condensate, of approximately 0.7 grams per second. This increase with time of pressurization almost certainly reflects the rate of heat loss from the gun presumably largely through the uninsulated lid.

The apparent discrepancy between the theoretical amount of steam required to pressurize the empty preheated gun, and the observed amount of steam required at time zero, was caused by heat loss from the empty gun during a delay of approximately 45 seconds between preheating and pressurizing. This delay was introduced in order to permit condensate from the cyclone (remaining from the preheating of the gun) to drain out completely before pressurization. Failure to have done so, would have resulted in the inclusion of the undrained part of this condensate, with the condensate to be measured in the experiment (i.e. from the steam used to pressurize the gun and to replace steam consumed by heat loss). Preheating of the gun was done before each experiment, by holding the otherwise empty gun under full steam pressure until the gun temperature ceased to rise. The gun was then emptied by firing the steam and condensate into the closed cyclone, with the ball valve of the cyclone open. The necessity of getting ride of this condensate from the cyclone, before determining each point along the curve, dictated the delay. In retrospect, it would have been better to have had no delay, to have pressurized the gun immediately after the preheating, and to have made no attempt to obtain experimental points for times shorter than 45 seconds. From Fig. 5 it can be seen that, if the lowermost curve were extrapolated back to a time of about 45 seconds before time zero, it would reach the theoretical weight of steam for pressurizing the empty gun.

The lower most curve in Fig. 5 was obtained without opening the lid of the gun. In practice, however, when a charge of wood is introduced into the gun, it is necessary to open the lid for this purpose after the gun has been preheated. The second-from-bottom curve of Fig. 5 was obtained by simulating the loading procedure, the lid being removed after the preheating, and left off for a time equal to that normally required in loading the gun. The gun was then pressurized and held under pressure for various times as in the obtaining of the lowermost curve described above. It can be seen that as a consequence of opening the gun, the curve was displaced upwards by an amount equivalent to about 40 grams of additional condensed steam.

The uppermost solid curve of Fig. 5 was obtained by adding to the second-from-bottom curve, the additional theoretical steam requirement for the heating of green aspenwood (MC 51.97%, OD basis) of equivalent dry weight 100 g. The observed experimental values for steam consumption, obtained when this weight of aspenwood was actually added to the gun, are shown by the points joined by the dotted line. Although these experimental points display excessive scatter (especially for times longer than one minute), those for times up to one minute lie about the uppermost solid surve.

A number of factors may have been instrumental in producing these unsatisfactory results. A significant amount of condensed steam (possibly of the order of 25 g) clings to the lower surface of the flat cyclone lid and around the inside of the cyclone. When the cyclone lid is not removed, as was the case when no wood was present, equal run-down of water from successive shots could be obtained by controlling the drainage time. When wood was present, removal of the lid was necessary for quantitative recovery of the wet exploded wood.

Washing with a weighed amount of water was required, followed by air-drying and moisture determinations on the air-dry solids. Sufficient care may not have been taken in cooling the cyclone thoroughly before opening. Significant moisture may have been lost by evaporation from the large area of warm metal during the recovery operation.

In addition, valve leakage, especially through the steam inlet valves was a problem in these measurements of steam consumption. These valves were replaced and the lower one was finally removed. Erratic leakage rates persisted, however, and contributed to moisture pick-up by the wood during loading. The main ball valve at the bottom of the gun leaked through to the cyclone only very slowly, when the gun was pressurized to 3.2 MPa (240^{o}C), and could be corrected in the calculation. The rate of this leakage was a constant 6 g per minute.

The possibilities of entrainment of water droplets in the steam entering the gun, or of the introduction of condensed water from the steam line, were considered. A suitable steam trap is readily available and should probably be installed just before the gun.

Attempts to repeat and extend the steam consumption experiments were frustrated by a series of intermittent problems with the thermostat, and with replacement thermostats, for the high-pressure boiler. In addition, prolonged down-times resulted from successive breakdowns of two high-pressure water pumps. A higher-pressure pump, capable of pumping against the maximum boiler pressure was finally obtained and installed, and a reliable supply of steam at constant pressure is now available. In the meantime, however, the steam consumption experiments were temporarily suspended.

STEAM-EXPLODED WOOD (SEW) - SIX SERIES

Six series of steam treatments of aspenwood for different times and under different conditions were made. Details of these treatments, and of the resulting steam-exploded wood, are shown in Tables 1-14 inclusive, and in Figures 6-10 inclusive.

SERIES 1

Treatments of Series 1 were made with green aspenwood chips in the 2-L gun at 240°C using the closed cyclone. Table 1 shows the decreasing yield of equivalent dry weight of steam-exploded wood (SEW), the increasing acidity, and the pronounced decrease in pentosan content, as the time of treatment was increased from 20 to 240 seconds. It can be seen that, at 240 seconds, less than one-fifth of the original pentosan of the wood remained.

SERIES 2

Treatments of Series 2 were essentially repeats of those of Series 1, except that more care was taken in the recovery of the exploded wood. The cyclone was cooled with a fine spray of water until cold, before opening. Table 2, however, shows that the recovery of equivalent dry weight of SEW was more scattered than that of Series 1. The higher moisture contents of SEW from Series 2 apparently reflect less loss of moisture on opening the cooled cyclone. Figure 6 shows a plot of percent recovery of SEW, on a logarithmic scale, against time of steam treatment. The points from Series 1 can be seen to lie close to the regression line drawn for all of the data of Series 1 and 2.

SERIES 3

Recovery From Gun

Series 3 was essentially a repeat of Series 1 and 2, except that the open cyclone was used and approximately 2.6 times as much green wood was used. (The amount of green wood used in Series 1 and 2 had been limited in order to avoid blowing the rupture disc of the closed cyclone). The yields of SEW recovered from the cyclone are listed in Table 3 and are plotted in Figure 7. As expected, these yields are similar to those obtained from the closed cyclone in Series 1 and 2.

Moisture Content of SEW - Implications For Steam Consumption

The only significant difference from Series 1 and 2 appears to be the lower moisture content of the SEW obtained from Series 3. A lower moisture content was to be expected because of the venting of the steam which filled the void volume of the gun and, much more importantly, because of a "flashing-off" of part of the liquid water present in the gun. This liquid water, consisting of the original moisture of the wood put into the gun, plus the condensed steam, left the gun at 240^{o}C and entered the open cyclone where the pressure was only one atmosphere. The liquid water, being far above its boiling point, boiled explosively until, presumably, its temperature and that of the exploded wood dropped to 100^{o}C.

If it is assumed that the amount of heat absorbed in the vaporization (calculated at 100°C) of liquid water from the wet exploding wood, is equal to the amount of heat given out by the cooling of the liquid water, the cooling of the wood, and the cooling of the steam in the void volume of the gun (all from 240° to 100°C), then it can be calculated that a maximum of one-third of the liquid water "flashes off". This value is a maximum because it assumes no absorption of heat in warming the air.

Assuming a charge of green wood of the weight and moisture content of that used in Series 3, and assuming the theoretical weight of steam required to heat this wet wood to 240°C and to pressurize the gun, the weights of liquid water and steam in the gun immediately before the firing were calculated. The amount of water flashing off was estimated, based on the assumptions described in the preceding paragraph, and the remaining theoretical minimum moisture content of the exploded wood to be expected, in the open cyclone, was calculated to be 145 percent of the weight of the oven-dry exploded wood. This value was a minimum because, if heat had been lost in warming air, this moisture content would have been larger. It can be seen, that the experimentally observed moisture content of 243 percent after 20 seconds, (Table 3), was much greater - not surprising in view of the continuing heat loss from the gun and the loss during loading shown in Fig. 5.

Working back from the observed 243 percent moisture content, it was calculated that the moisture content immediately before the gun was fired must have been 363 percent. (In this case this value is a maximum. It would have been smaller if air had been heated during the "flash-off.") This calculated value, before firing, is roughly similar to the badly scattered experimental values of 309, 375 and 338 percent (Table 2), observed after 20, 40 and 60 seconds, respectively, after firing into the closed cyclone in Series 2, where no steam could escape and where slightly dryer wood was used.

It is concluded, therefore, that the moisture contents of the exploded wood obtained in Series 3 with the open cyclone are consistent, within the poor accuracy of the data, with the moisture contents obtained in Series 2 with the closed cyclone. The steam consumptions, at least for the shorter times of steam treatment, were apparently similar, the lower moisture contents of Series 3 being the result of the "flashing-off" of the expected amount of steam through the open cyclone.

The moisture contents of Series 2 with the closed cyclone are, furthermore, consistent with the uppermost solid curve of Fig. 5 which was described earlier. This curve, consists of the observed amount of steam required to pressurize the otherwise empty gun and replace heat loss from the gun with time, plus the observed additional steam consumed in replacing heat lost on opening the

lid of the preheated gun to simulate the loading operation, plus the amount of steam theoretically required to heat the wet wood of the charge to steam temperature. Accordingly, if the amount of original moisture present, in the wet charge of wood loaded into the gun, is added to this uppermost solid curve of Fig. 5, the observed moisture contents from Series 2, listed in Table 2 for the closed cyclone, are obtained - again, within the poor accuracy of the data. The calculated moisture content for the 20 second treatment, for example, was 318 percent (oven-dry basis), compared with 309 percent in Table 2. These observations, therefore, indicate that much of the observed greater-than-theoretical over-all steam consumption for the process could be explained by the continuous heat loss from the gun and by heat loss during the loading operation.

It is noteworthy that a similar steam consumption was apparently experienced with green aspenwood in the larger Iotech gun. Accordingly, Foody (1982) has reported that, after a 20-second treatment at a steam pressure equivalent to 248^{o}C, steam-exploded wood of moisture content 72.12 percent (wet basis) was obtained. This moisture content is equivalent to 258.7 percent on an oven-dry basis and, should be equivalent to approximately 246 percent if the steam temperature had been lower, at 240^{o}C, as in the present work. This adjusted Iotech moisture content of 246 percent compares fortuitously closely with the moisture content of 243 percent obtained in the present work after 20-seconds (Table 3).

Yield and Pentosan Content of Water-Soluble Fraction

The amount of water-solubles, extractable from the SEW of Series 3 (by water at room temperature), are listed in Table 4, together with the pentosan contents of these water-solubles. Details of the extraction procedure are included as a footnote to this Table. The maximum amount of pentosan was found in the extract from the 80-second steam treatment and represented 51.6 percent of the original pentosan present in the wood loaded into the gun. After 80 seconds at 240°C, the amount of soluble pentosan decreased until, after 240 seconds, only 16.1 percent of the pentosan of the original wood remained in the water-soluble fraction. This decrease is not surprising in view of the continuous destruction of pentosan known to be occurring throughout all the time of the steam treatments, as shown in Table 1. The 16.1 percent of original pentosan, remaining in the water-solubles after 240 seconds, is in reasonable agreement with the pentosan content of unextracted SEW obtained after 240 seconds in Series 1. The 3.54 percent reported in Table 1 is equivalent to 19.3 percent of the original pentosan and includes both soluble and insoluble pentosan.

Water Insoluble Fraction

After removal of the 1st water-extract and washings, as described above and in Table 4, the undissolved fractions remaining were extracted twice more with water to remove any residual traces of solubles. The analyses of the resulting, thoroughly water-washed,

steam-exploded wood (WSEW) remaining, are listed in Table 5. It can be seen that the pentosan content had been reduced from 18.35 percent of the wood loaded into the gun, to 7.56 percent of the washed steam-exploded wood, after only 20 seconds at 240°C followed by explosion and water extraction. This content of residual pentosan decreased with increasing time of steam treatment until, after 240 seconds it was only 1.01 percent of the WSEW. A summation, for each time of treatment, of the water-soluble pentosan from Table 4 with the water-insoluble pentosan from Table 5 (both calculated to an original wood basis) gave totals which were, on the average, 1.3 percent lower than the total pentosan contents listed in Table 1 for whole exploded wood in Series 1. These slightly lower summations possibly reflect the loss of small additional amounts of pentosan in the second and third extractions.

Table 5 also shows that the total apparent lignin content of the WSEW increased from 25.9 to 35.7 percent, as the time was increased from 20 to 240 seconds. Much of this increase in apparent lignin resulted from the fact that most of the true lignin of the original wood survived the steam treatment and remained insoluble in water, whereas the base weight decreased as a result of an over-all weight loss of the exploded wood and a solubilization of part of the carbohydrate (principally pentosan). The decrease in base weight, however, was insufficient to explain all of the increase in apparent-lignin content. Accordingly, when these lignin analyses were recalculated to an original-wood basis, the values increased steadily from 20.2 percent at 20 seconds,

to 24.9 percent at 240 seconds. The increase presumably resulted from increasing amounts of accumulated water-insoluble degradation products ("pseudolignin") from the pentosan.

The glucan analyses, reported in Table 5, and throughout this report, were done as indicated in a footnote to Table 5. Although all glucan values are corrected for pentosan, they are not corrected for mannan because analytical values for the latter were not determined. Glucan values listed, therefore, are actually glucan plus mannan. The resulting errors in the glucan values, however, are small because even in the original aspenwood the mannan content was only about 2 percent. No clearly discernable trend is evident in the glucan analyses of Table 5, the entire range being only 3.5 percent. The fact, however, that the base weight decreased with increasing time, dictates that the glucan content should actually have increased, unless some were lost or, more likely, were solubilized. In any case the calculated decrease in base weight, between 20 and 240 seconds, was only 10.4 percent, the decrease in recovery of steam-exploded wood being partly balanced by the increase in the water-insoluble fraction at longer times. The actual decrease may have been somewhat less than 10.4 percent because of greater mechanical losses encountered during recovery of steam-exploded wood from the cyclone after longer times of treatment. Nevertheless, assuming the observed recovery from the cyclone, and the observed amount of water-solubles, the amount of glucan in the water-washed steam-exploded wood was calculated to decrease from 45.5 percent at 20 seconds, to 40.3 percent

at 240 seconds, both values being based on the original weight of wood.

These values may be compared with Timell's (1967) analysis of solvent-extracted aspenwood which gives the cellulose content as 48 percent, and the glucomannan content as 3 percent. On an extractives-containing basis, comparable to that in the present work, these Timell values become 46.6 and 2.9 respectively, the latter of which is equivalent to 2.0 percent of mannan. It should be remembered that, at the longer times, part of the original cellulose and much of the original glucomannan (which is amorphous, is more easily solubilized, and is more durable than pentosan) would be expected to be in the water-soluble fraction. Because it is unlikely that a steam treatment under these conditions would exceed 120 seconds, at which time the glucan content of the WSEW was calculated to be 43.5 percent (original wood basis), it is unlikely that cellulose destruction was greater than 2 or 3 percent.

A summation of the pentosan, total apparent lignin, and glucan contents of the WSEW, is given in Table 5. These components totalled from 91.8 to 95.5 percent and thus accounted for most of the material present. A 100 percent summation was not expected because small amounts of other materials were known to be present. For example, much of the original extractives, constituting 3 percent of the wood, should still be present. Very small amounts of acetyl, not hydrolyzed in the steam treatment, and small amounts of uronic acid in the residual xylan, together probably account for about

0.6 percent in the 20-second product.

SERIES 4

Series 4, unlike all other series in this report, was done with air-dry rather than with green wood, and was done in the small 200-ml Forintek steam gun rather than in the 2-litre Forintek gun. Other differences were that the air-dry aspenwood used was in the form of thin wafers 0.5-0.75 mm thick, rather than in the form of 3.2 mm chips cut across the grain, and that the air-dry wafers were slightly higher in pentosan content (20.31 percent vs 18.35 percent).

The yields of steam-exploded wood recovered from the cyclone of the 200-ml gun are listed in Table 6. These yields, obtained from the air-dry wafers, can be seen, generally, to be a few percent lower than the comparable yields reported in Table 3 for green (i.e. wet) chips in the larger gun. The lower yields, although they could indicate a more rapid heating of the thin wafers, might instead merely reflect a greater mechanical loss of exploded wood in the relatively larger cyclone of the 200-ml gun.

The yields, pentosan contents, and apparent-lignin contents of the water-soluble fractions obtained from the air-dry wafers, are also listed in Table 6. Comparison of these dry-wafer data, with comparable data in Table 4 for the wet chips used in Series 3, shows the results to be roughly similar, for times up to about

60 seconds, the slightly lower yields of water-solubles from the dry wafers being somewhat more than balanced by slightly higher pentosan analyses. If, however, the pentosan contents of the water solubles, in both cases, are expressed as percentages of the original amount of pentosan in the wood put into the guns (to avoid the influence of the higher original pentosan content of the wafers), then the two sets of data for solubilization of pentosan appear virtually identical for times up to 60 seconds. This similarity can be seen more readily by comparing the two lower curves shown in Fig. 8. The apparently more rapid decline in the amount of soluble pentosan, evident in Fig. 8 for the dry wood after 60 seconds, may be real, and may reflect the more rapid decline in the overall yield of exploded wood discussed above, or it may merely result from experimental error.

The lignin content of the water-solubles fraction (16.2 percent) after 15 seconds, when expressed on an original wood basis, corresponds to 2.5 percent. Interestingly, this represents about 12 percent of the original lignin of the wood, or about the same fraction as that normally present in the Klason lignin filtrate as "acid-soluble lignin" during lignin analysis of aspenwood.

The pentosan and apparent lignin analyses, of the WSEW remaining as the water-insoluble fractions from the dry wafers, are listed in Table 7. Comparison of these pentosan analyses based on the WSEW, with the corresponding values in Table 5

obtained from the originally wet chips, shows the two sets of data to be roughly similar. Again, by removing the effect caused by the different original pentosan contents of the dry wafers and wet chips, the two sets of data fell along one curve, within the accuracy of the data, as shown in Fig. 9. In this case, the dry-wafer data were adjusted downward by multiplication by 18.35/20.31, i.e. by the ratio of the pentosan analysis of the original wet chips to that of the dry wafers.

The similarity of the results obtained in the solubilization of pentosan during steam treatment of dry or of wet wood, as measured by pentosan content of the water-soluble material produced, and by the complementary pentosan content of the water-insoluble material remaining undissolved, indicate that air-dry wood could be used and thereby effect a substantial saving of high-pressure steam. The comparison, however, should be repeated under more comparable conditions, using the same chips (wet and dry), in the same gun.

It is possible that the similar results were obtained because the particle size was small in both cases, permitting similar rapid heat transfer into the wood. If liquid water is necessary, perhaps it was provided rapidly by the condensing saturated steam as it contacted the initially cold wood particles. In this case, both wet and dry starting materials would have been effectively wet.

SERIES 5

The steam treatments of Series 5 were done on aspenwood chips which had been vacuum impregnated with 0.20 percent sulfuric acid, as described in the footnote to Table 8. The steam temperature was reduced to 220°C, from the 240°C used in all of the other series; otherwise the conditions were the same as those of Series 3 with the open cyclone. The results are described in Tables 8,9, 10 and 11.

The slightly lower recoveries of SEW shown in Table 8, compared with those of Table 3 from wood not impregnated with acid, were lower, partly because of some mechanical loss in the blotting operation to remove excess impregnating solution, and partly because of a loss of water-soluble material from the chips during impregnation and soaking. The soluble fraction of untreated aspenwood is typically 1.5 percent in cold water, and 3.0 percent in hot water.

The pentosan, apparent-lignin, and glucan analyses of the whole exploded wood, before extraction with water, are listed in Table 9. A comparison of these pentosan analyses (acid-impregnated, 220°C) with the pentosan analyses of Table 1 (not-acid impregnated, 240°C), both series calculated on an SEW basis, is made in Fig. 10. These two sets of pentosan analyses, plotted in Fig. 10, both lie close to the upper line drawn in this figure. This similarity, before water-washing, shows that the accelerating effect of the acid-impregnation on the processes of pentosan destruction, was closely balanced by the slowing effect of the lower temprature selected for this purpose. The rate of over-all pentosan destruction, in acid-impregnated wood at 220°C, was essentially equal to that of unimpregnated wood at 240°C, thereby facilitating a meaningful comparison of the relative extents of pentosan solubilization.

The yields of water solubles, and the pentosan content of these water solubles, (acid-impregnated, 220°C) are shown in Table 10, and may be compared with the yields and pentosan contents of the water solubles (not acid-impregnated, 240°C) shown in Table 4. A comparison is made in Fig. 8 of the amounts of soluble pentosan produced. The uppermost curve (acid-impregnated, 220°C) lies substantially above the middle curve (not acid impregnated, 240°C), showing a significantly greater extent of maximum pentosan solubilization and a much more rapid attainment of this maximum.

The complementary analyses of the water-washed steam-exploded wood remaining after removal of the water-solubles (acid-impregnated, 220°C) are listed in Table 11, and may be compared with the comparable data in Table 5 for the not-acid-impregnated series at 240°C. This comparison of the pentosan analyses is made in Fig. 10. It can be seen from the two lowest curves in this figure, that a much more complete and rapid removal of pentosan was obtained with the acid-impregnated wood even though the over-all destruction of pentosan (shown by the uppermost curve of this figure was essentially the same).

The glucan contents of the over-all SEW (acid-impregnated, 220°C) listed in Table 9, before water extraction, show no indication of destruction of cellulose even after the longest treatment (80 seconds). The values, listed on an original-wood basis, vary from 49.9 to 51.6 percent with no evident trend. These can be compared with Timell's values for aspenwood of 48 percent for cellulose and 3 percent for glucomannan, on an extracted-wood basis, (equivalent to 46.6 and 2.9

percent, respectively, on an extractives-containing, original-wood basis). Although not destroyed, a small part of the cellulose of the acid-impregnated SEW, may have been solubilized at the longer times under these conditions. Accordingly, in Table 11, the glucan content of these exploded-wood products, after water washing, appears to decrease with increasing time even before reduction to an original-wood basis. In practice, however, under these conditions, it is unlikely that a treatment of much more than 20 seconds would be required.

SERIES 6

Series 6 was essentially a repeat of Series 5 except that the normal steam temperature of 240°C was used (instead of the reduced temperature of 220°C). The results are shown in Tables 12, 13 and 14. Comparison of these tables with those for Series 5 (i.e. Tables 8 to 11, inclusive) shows, as expected, much faster changes in the chemical composition of the products at the higher temperature. The rate of destruction of pentosan appears to have been 3 or 4 times faster, in rough agreement with the 20°C difference in temperature. Solubilization, and removal of pentosan by water extraction, appear to have been increased even more, a residual pentosan content of 0.47 percent after 10 seconds at 240°C being similar to the 0.49 percent after 80 seconds at 220°C. Comparisons at levels below 1 percent, however, are probably not significant.

Essentially all of the pentosan in all of the treatments of Series 5 and 6 was either solubilized and extracted in the water wash, or was destroyed.

Although no evidence of cellulose destruction was apparent from the glucan analyses in Series 5, the decreasing glucan analyses of the exploded wood samples of Series 6 before water extraction (Table 13), and even before adjustment to an original-wood basis, suggests some destruction. The analytical values of 57.8 and 56.2 percent, however, for treatments of 10 and 20 seconds, respectively, are not sufficiently different to be convincing, and cannot reliably be converted to an original wood basis because of apparently unreliable data for recovery from the gun in this series (Table 12). The only evidence for destruction of cellulose rests with the one low glucan analysis of 52.7 percent for the 40-second treatment, and the fact that the true yield of exploded wood, from this longest treatment, must have been no greater than the true yields from the two shorter treatments which gave significantly higher analyses. The more pronounced decrease in glucan content after water washing, shown in Table 14, even without adjusting to an original wood basis, supports the analogous evidence of Table 11 for Series 5, that some of the glucan was solubilized.

CORRELATION OF DATA FROM FORINTEK AND IOTECH GUNS

The easiest chemical process to follow during steam treatment is the destruction of the pentosan. Therefore, in order to compare the relative extent of the changes occurring in the 2-litre Forintek gun, with those reported by Foody (1980, 1982) to be occurring in the larger Iotech gun, the curve shown in Fig. 11 was drawn. In order to prepare this curve, pentosan analyses reported by Foody were plotted against the time, at the steam pressure equivalent to 250°C. A similar plot was prepared of the pentosan analyses obtained with the 2-litre Forintek gun against the time at 240°C. Smoothed curves were drawn through these data, and were adjusted to a uniform initial pentosan content in the original wood used. A series of pentosan contents were arbitrarily selected over the range of interest, and the corresponding equivalent times were read from the two smoothed curves and were plotted one against the other to obtain Fig. 11. The smooth curve of Fig. 11 therefore shows the time required in the 2-litre Forintek gun at 240°C, to produce the same degree of destruction of pentosan as that produced after any time of interest in the Iotech gun.

A significant feature of this curve is its pronounced curvature at short times. At times of up to 35 seconds, the extent of pentosan destruction, and presumably of all other processes, was actually further advanced in the Forintek gun, even though the steam temperature was 10°C lower than that in the Iotech gun. After about 50 seconds in the Iotech gun, however, the continuing

curvature led to a rate of pentosan destruction approximately double that in the Forintek gun, more in accord with the $10^{o}C$ difference in steam temperature.

These differences indicate, not that one gun is better than the other, but rather that for some reason the charge of wood in the Iotech gun rose more slowly in temperature, despite the hotter steam. It is suggested that the reason was that a larger chip size was used by Iotech, and that because of slower heat flow into the centres of the larger chips, the centres were under-cooked relative to the outer surfaces. The resulting heterogeneity of the exploded wood, as to degree of under- or over-cooking, could be important, if pronounced, because the observed optimum time of treatment would then be only the best over-all average, and because over-cooking was found to produce more fermentation inhibitors. The rate of temperature-rise inside simulated wood chips was therefore measured.

TEMPERATURE RISE INSIDE WOOD SPECIMENS

The temperatures, 3.2 or 6.4 mm beneath the surface of freshly cut green wood specimens of different geometrical shapes and sizes, were measured, while these initially cold specimens were heated in saturated steam at a constant temperature of 240°C. The specimens were heated, one at a time, in the middle of the 2-litre steam gun, out of contact with the metal of the gun. They were suspended in this position in the otherwise empty preheated gun, by means of the 6.4 mm-diameter thermocouple probe which extended vertically downward through the lid. The end of the probe was inserted, in turn, into each specimen through a hole of diameter 6.4 mm, drilled to a depth just 3.2 or 6.4 mm short of the opposite face of the specimen, as shown in Fig. 12.

The sensitive tip of the probe was in close contact with the wood at the bottom of the hole in each case, held there by the tight fit of the probe in the hole. Heat flowed to the sensitive tip of the probe through the remaining thin layer of green wood of thickness either 3.2 or 6.4 mm. Specimens, numbered 1-5 inclusive, were cylindrical in shape, like wooden dowels. Of these, specimens 1, 2, and 3 were cut on a lathe to an outside diameter of 12.7 mm, so that the probe was surrounded on the sides, as well as on the tip, by a uniform layer of wood of thickness 3.2 mm. Specimens 4 and 5, of diameter 19.1 mm, correspondingly surrounded the probe with a layer of wood of thickness 6.4 mm (i.e. twice the thickness of that in specimens 1, 2 and 3).

The fibre, or grain direction, in all of these dowel-shaped specimens was parallel to the axis of the dowel. It was not feasible to prepare and use similarly shaped specimens, with the direction of the grain at right angles to the axis. Specimens 6 and 7, of rectangular shape, were accordingly prepared to detect any major difference in the rate of heat flow along or across the grain. They were sufficiently large to avoid any major interference in the across-the-grain rate measurement with Specimen 6, by a possibly rapid flow through the end grain to the probe.

The resulting temperatures inside the specimens, as indicated by the probes, are plotted in Fig. 12. As expected, the temperatures tend to approach, asymptotically, the constant steam temperature of 240^{o}C, which was simultaneously measured with a second thermocouple probe. It is noteworthy that, in a few cases, as the steam temperature was approached, shrinkage of the specimen eventually caused it to slip from the probe and fall to the bottom of the gun - an event which was marked by a sudden rise in the probe temperature, to that of the steam, as measured by the second probe. The identical readings of steam temperature by the two probes indicates the absence of a constant discrepancy between the two probes, which could have been significant as the steam temperature was approached.

From Fig. 12 it can be seen that the probe temperature, at a distance of 3.2 mm inside Dowel No. 2, reached 230^{o}C (i.e. 10^{o}C below the steam temperature) after 1.54 minutes in the steam

of temperature 240°C. A slight extension of the curve for Dowel No. 4 shows that, at twice this distance (i.e. 6.4 mm) inside Dowel No. 4, this temperature of 230°C was reached after twice this time (i.e. after 3.08 minutes). Both dowels started at the same temperature. Although this precise agreement was fortuitous, some confidence can be placed in the data because it shows twice as long a time for twice as long a heat path.

Some concern was felt, that the response time of the probe (described above in the section on the gun and its instrumentation) might be too long, and that the probe temperature might lag significantly behind the rapidly rising temperature of the wood. Assuming the response time specified by the manufacturer, however, it was calculated that the lag had introduced an error of no more than 3 percent in the measured time of 1.54 minutes for the probe site in Dowel No. 2 to reach 230°C. At lower temperatures where the temperature curve was rising more steeply, the lag-induced error was, of course, greater. For the purpose, of estimating the time required for the interior of a chip to reach a temperature close to the steam temperature, the lag-induced error is acceptable.

The rates of rise of temperature observed with Blocks No. 6 and 7 are, of course, much lower because of the large heat sink provided by the interior of the blocks. In order to observe the rates better, the logarithm of the difference in temperature between the steam and the probe were plotted against the time in Fig. 13 for the dowels, and in Fig. 14 for the blocks. Ideally, because the temperature difference should decrease at a rate proportional to the temperature difference, the logarithmic

plot should give a straight line. In general, it can be seen that the data were reasonably well described by straight lines over a large part of the heating period. Slopes of regression lines for Dowels No. 1 and 2, over comparable ranges, were -1.67 and -1.69 minutes^{-1}, compared with -0.88 and -0.84 minutes $^{-1}$ for No. 3 and 4, the latter pair of values (for double the thickness) being approximately one-half as great as the former pair. Comparable slopes for Blocks No. 6 and 7 were -0.35 and -0.32 minutes^{-1} indicating very little difference in heat flow along the grain as compared with across the grain under these conditions. The slightly greater rate (8 percent greater) for Block No. 7, even though its volume was 14 percent greater than that of No. 7, suggests that, if anything, the flow along the grain was slightly faster than that across the grain.

The principal significance of these temperature measurements is that they show that the centres of all except very thin chips must be under-cooked or, conversely, the outsides must be over-cooked. The heat flow along the grain, through a thickness of only 3.2 mm of green wood, was so slow that even after 1.5 minutes the temperature was still 10^{o}C below steam temperature. Presumably a chip 3.2 mm thick would be heated from both sides and its centre would reach the temperature (10^{o}C below steam temperature) in half of this time, i.e. after 45 seconds. Nevertheless, this is comparable to the time of the whole steam treatment when acid is used, and means that the rate of the chemical processes occuring in the centre of the chip only approach one-half the rate of those at the outer surface, near the end of the treatment.

Evidence of the resulting heterogeneity of the steam-exploded wood produced has been seen in the appearance of the material itself. A significant fraction, composed of large fibre bundles, accompanies the completely defibred material. Furthermore, an examination by electron microscopy of slices, cut from inside Block No. 6 at varying distances from the lower face, showed a pronounced gradation in appearance. Slices, taken, for example, from a distance of only 1 or 2 mm in from the lower face, showed much greater effects of the heat than did those cut at the probe distance of 6.4 mm. In principle, therefore, the most homogeneous product should be producible from the smallest chip-size consistent with good steam penetration between the chips.

The specimens (dowels and blocks) used in these experiments were not subjected to explosive decompression at the end of the steam treatments. Instead, the steam was bled out, through a valve in the lid of the gun, permitting retrieval of the specimens.

All of the steam treatments of the six series described in this interim report were done with chips 3.2 mm thick. These chips were cut so that this smallest dimension was in the fibre direction. In some additional experiments in the 2-litre gun, not presented here in detail, similarly cut chips of thickness 6.4 mm and also fines sieved from the 3.2 mm chips were used. In no case did steam penetration between the chips of the charge appear to be restricted. The temperature at the centre of the charge

(as measured by the long axially-mounted probe extended down between the chips to the centre of the charge) rose to the inlet-steam temperature in a few seconds.

PROCESS OF PENTOSAN DESTRUCTION

It has generally been believed that the principal process occurring in the steam gun is hydrolysis. This hydrolysis was believed to be responsible for reducing the degree of polymerization of the pentosan component, and for making much of the pentosan water-soluble. Xylose, the ultimate product of pentosan hydrolysis was then believed to be dehydrated to furfural, part of which condensed with the lignin. The increase in the weight of the original true lignin was therefore the "pseudolignin". From the data collected in the present study, however, it now appears likely that the principal process occurring in the steam gun is pyrolysis.

In Fig. 15, the pentosan lost, the pseudolignin formed, and the furfural formed and retained are plotted against time at 240^{o}C in the steam gun. From this figure, it can be seen that, over a large part of the course of pentosan decomposition, pseudolignin was forming at an almost constant rate, even though, in the early stages, essentially no xylose monomer and essentially no furfural were present. Later, by the time the concentrations of xylose and of furfural had increased over six fold, the rate of pseudolignin formation still remained virtually unchanged, indicating an independant process. If the pseudolignin had been forming from the furfural, the rate of pseudolignin formation would have been expected to have increased with increasing furfural concentration. It is suggested that the pseudolignin is a pyrolysis product, formed directly from the pentosan, without passing through the furfural stage.

Elementary analysis of aspenwood (not acid-impregnated), before and after steam explosion, showed that the ratio of oxygen/carbon decreased from 0.88 to 0.80 as a result of the steam treatment. The ratio of oxygen/hydrogen, at the same time, dropped from 7.4 to 6.9. Expressed on an original-wood basis, significant losses in both carbon and oxygen were evident, with the greatest loss shown by the oxygen. These changes were qualitatively in accordance with the loss of both carbon dioxide and water - both of which are products of pyrolysis.

It is recognized, however, that if the lost pentosan had been lost by hydrolysis to xylose and dehydration to furfural, and if the furfural had been retained or had been converted to pseudolignin, then the lost material would have had the composition of water. Loss of part of the furfural, by volatilization in the steam flashing off, (when cosidered together with the water formed with the furfural) would have been equivalent to losing material of the composition of pentosan.

In order to obtain more direct evidence of pyrolysis in the steam gun, charges of green aspenwood, of equivalent oven-dry weight 250 grams, were heated in the 2-litre gun at 240^{o}C for periods of either 1 or 2 minutes. Permanent gases and steam present in the gun after these periods of time were bled-off through a 6.4 mm valve in the top of the gun and were passed through a water-cooled copper condenser. The permanent gases were then collected by downward displacement of acidified water and were analyzed by gas chromatography. The composition after 1 minute is shown in Table 15. An essentially similar composition

was found after 2 minutes, in which case the volume of gas obtained was approximately double that after 1 minute.

This composition, consisting almost entirely of carbon dioxide, is characteristic of the early stage of low-temperature pyrolysis (Merritt and White, 1943). The weight of carbon dioxide found after 2 minutes at 240°C was equal to 2 percent of the weight of the wood, or to 10 percent of the weight of the original pentosan. It appears, therefore that significant pyrolysis occurs in the gun under these conditions.

A number of parallels can be found between steam explosion and partial pyrolysis at atmospheric pressure (Merritt and White 1943). Red oak, heated in an atmosphere of steam at atmospheric pressure, underwent partial pyrolysis at temperatures below 180°C. At 240°C the pentosans were almost completely destroyed. The elemental analysis underwent similar, although more extreme changes, including a pronounced decrease in oxygen. The lignin content apparently increased, and it was concluded that some material was becoming insoluble under the conditions of the Klason lignin analysis. It is also noteworthy that superheated steam at atmospheric pressure acted partly as an inert gas but also as a reactive agent, and that, although longer treatment times were used than in the comparable steam explosion, these times could be greatly reduced when smaller particle sizes of wood were used.

If, therefore, pyrolysis is a major process in the gun, and if this pyrolysis is the desired process in pentosan break-down and solubilization, and is not merely an undesirable side reaction, then it should be worthwhile to examine superheated steam at atmospheric pressure. If solubilization of the pentosan can be achieved, then susceptibility to enzymatic hydrolysis should be examined.

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TABLE 1. (Series 1[1], Closed Cyclone, 2-L Gun, 240°C)
Recovery From Gun, and Destruction of Pentosan

Time (s)	Yield of OD SEW[2] (% of original wood)	MC[3] of SEW from gun (%, OD basis)	pH of wet SEW	Pentosan in SEW	
				(% of SEW)	(% of original wood)
20	95.6	269	3.58	17.08	16.33
40	95.2	237	3.58	15.76	15.01
80	90.8	248	3.49	13.38	12.15
120	87.0	356	3.35	11.42	9.94
180	83.8	322	3.29	7.93	6.64
240	79.7	363	-	4.44	3.54

[1] Green aspenwood chips; moisture content 51.97% (wet basis); pentosan content 18.35%; 3.2 mm thick in fiber direction; from freshly felled trees; chips stored in deep freeze; weight loaded into gun 208.2 g wet = 100.0 g oven-dry (OD)

[2] Equivalent oven-dry (OD) steam-exploded wood (SEW) recovered from gun, as percent of dry weight of original wood put into gun

[3] MC - moisture content of exploded product as percent of oven-dry product

TABLE 2. (Series 2[1], Closed Cyclone, 2-L Gun, 240°C, Repeat of Series 1)

Recovery From Gun

Time (s)	Yield of OD SEW (% of original wood)	MC[2] of SEW from gun (%, OD basis)
20	96.2	309
40	91.8	375
60	95.2	338
80	93.3	382
120	86.8	383
180	83.2	419
240	80.8	320

[1] See footnotes of Table 1

[2] Cyclone cooled before opening, by spraying outside with cold water, to ensure complete condensation of steam

TABLE 3. (Series 3[1], Open Cyclone, 2-L Gun, 240°C)

Recovery From Gun

Time (s)	Yield of OD SEW (% of original wood)	MC of SEW from gun (%, OD basis)
20	95.5	243
40	94.8	250
80	93.3	231
120	89.9	285
180	84.2	271
240	78.5	286

[1] See footnotes of Table 1, except that a greater amount of wood was loaded into the gun in this Series 3. Each charge consisted of 547.6 g wet wood ≡ 256.1 g oven-dry wood.

TABLE 4. (Series 3[1] continued, Open Cyclone, 2-L Gun, 240°C)

Amount, and Pentosan Content of Water-Solubles Fraction[2] of Steam-Exploded Wood

Time (s)	Yield of water-solubles (% of SEW)	Yield of water-solubles (% of original wood)	Pentosan content of water-solubles (% of water-solubles)	Pentosan content of water-solubles (% of SEW)	Pentosan content of water-solubles (% of original wood)	Pentosan content of water-solubles (% of original pentosan of wood)
20	19.4	18.6	45.1	8.76	8.37	45.6
40	20.1	19.1	48.9	9.83	9.32	50.8
80	19.4	18.1	52.2	10.14	9.46	51.6
120	20.1	18.1	40.8	8.19	7.37	40.2
180	17.3	14.6	29.5	5.10	4.30	23.4
240	16.1	12.6	23.4	3.76	2.95	16.1

[1] See footnotes of Tables 1 and 3

[2] SEW was extracted (ie washed) with water at room temperature by vigorous mechanical stirring of a slurry (roughly 5 percent consistency) for 2 hours, followed by filtration and rinsing of the insoluble fraction on the filter. The filtrate and rinsings were evaporated under reduced pressure at 40°C and were dried under high vacuum.

TABLE 5. (Series 3[1] continued, Open Cyclone, 2-L Gun, 240°C)

Pentosan, Lignin and Glucan Contents of Water-Washed Steam-Exploded Wood (WSEW), (i.e. of the Water-Insoluble Fraction)[2]

Time (s)	Pentosan content (% of WSEW)	Apparent lignin content			Glucan[3] (% of WSEW)	Sum of: pentosan lignin glucan (% of WSEW)
		Klason lignin (% of WSEW)	Acid-soluble lignin (% of WSEW)	Total (% of WSEW)		
20	7.56	23.9	2.0	25.9	58.5	91.9
40	4.77	26.4	2.4	28.8	59.8	93.3
80	2.48	29.1	1.8	30.9	58.4	91.8
120	1.99	30.6	1.9	32.5	61.0	95.5
180	1.05	32.4	2.3	34.7	57.5	93.2
240	1.01	33.0	2.7	35.7	57.8	94.7

[1] See footnotes of Tables 1 and 3

[2] Water extraction described in Footnote 2 of Table 4 for separation of bulk of water solubles. In addition, the water-insoluble fraction obtained was given two more extractions (the water extracts from which were discarded)

[3] Modified anthrone procedure in which the contribution by pentosan is minimized, and a correction for the residual pentosan is made, based on the pentosan analysis

TABLE 6. (Series 4[1], Open Cyclone, 200-mL Gun, Dry Wood, 240°C)

Recovery of Steam-Exploded Wood from Gun, and Amount and Analysis of Water-Soluble Fraction, when Wood Loaded into Gun was Air-Dry instead of Wet

Time (s)	Yield of OD SEW (% of original wood)	Water-soluble fraction (WS) of steam-exploded wood (SEW)				
		Yield of WS (% of original wood)	Pentosan Content of WS			Apparent lignin content of WS (% of WS)
			(% of WS)	(% of original wood)	(% of original pentosan of wood)	
15	96.0	15.6	53.0	8.3	40.7	16.2
30	94.2	18.0	53.8	9.7	47.8	14.2
60	89.0	19.4	54.3	10.5	51.9	-
90	87.8	20.2	45.5	9.2	45.3	-
120	85.8	18.4	41.1	7.6	37.2	-

[1] Air-dry aspenwood wafers, 0.5-0.75 mm thick; pentosan content 20.31%; small gun (200 mL) firing into relatively large open cyclone

TABLE 7. (Series 4[1], Open Cyclone, 200-mL Gun, Dry Wood, 240°C)

Pentosan and Lignin Contents of Water-Washed Steam-Exploded Wood (WSEW), i.e. of the Water-Insoluble Fraction (WI)

Time (s)	Pentosan content of WSEW			Apparent lignin content (% of WSEW)
	(% of WSEW)	(% of original wood)	(% of original pentosan of wood)	
15	11.4	9.0	44.3	24.0
30	8.0	6.0	29.5	23.8
60	4.0	2.8	13.8	-
90	2.2	1.5	7.4	-
120	1.5	1.0	4.9	-

[1] See footnote of Table 6

TABLE 8. (Series 5[1], Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 220°C)

Recovery from Gun, and Water Extraction

Time (s)	Yield of OD SEW (% of original wood)	MC of SEW (%, OD basis)	Volume of water extract (mL per gram of OD original wood)	pH of water extract
10	88.6	260	13.2	3.20
20	88.1	275	15.4	2.35
40	85.5	284	16.2	2.83
80	91.0	258	15.6	2.84

[1] Green aspenwood chips (3.000 kg wet weight ≡ 1.441 kg dry weight) were vacuum impregnated with 0.20 percent sulfuric acid. After standing in the acid solution for 64 hours at 50°C the chips were blotted between towels. Weight of wet impregnated chips obtained was 3.97 kg. Weight of wet impregnated chips loaded into gun was 397.0 g ≡ 144.1 g oven-dry original wood before impregnation. Impregnated chips stored, frozen, in deep freeze for few days before use.

TABLE 9. (Series 5[1] continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 220°C)

Analysis of Whole Steam-Exploded Wood[2], Before Extraction With Water

Time (s)	Pentosan		Apparent lignin			Glucan[3]		Total of: pentosan lignin glucan
	(% of SEW)	(% of original wood)	Klason lignin (% of SEW)	Acid soluble lignin (% of SEW)	Total lignin (% of SEW)	(% of SEW)	(% of original wood)	(% of SEW)
10	17.24	15.28	21.8	1.8	23.6	56.4	49.9	97.0
20	16.99	14.98	21.5	1.9	23.5	58.5	51.6	98.7
40	15.68	13.40	22.4	2.1	24.5	58.0	49.6	97.7
80	14.35	13.06	22.9	2.3	25.2	54.9	50.0	94.3

[1] See footnote of Table 8

[2] Sulfuric acid, in sample of wet SEW for analysis, neutralized with standard sodium hydroxide before air-drying; all analyses corrected to salt-free basis

[3] See footnote 3, of Table 5

TABLE 10. (Series 5[1] continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 220°C)

Analysis of Water Solubles (WS) of Water Extract

Time (s)	Yield of water solubles[2] (% of original wood)	Pentosan content of water solubles (% of WS)	(% of original wood)	(% of original pentosan of wood)
10	23.3	48.8	11.4	62.1
20	25.0	47.6	11.9	64.9
40	26.2	42.9	11.2	61.3
80	25.0	39.1	9.8	53.2

[1] See footnote of Table 8

[2] Sulfuric acid in extract neutralized with standard sodium hydroxide; extract evaporated at 40°C under vacuum and dried to constant weight under high vacuum; yield corrected for salt of neutralization

TABLE 11. (Series 5[1] continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 220°C)

Analysis of Water-Washed Steam-Exploded Wood (WSEW)
(i.e. the Water-Insoluble Fraction of Steam-Exploded Wood)

Time (s)	Pentosan (% of WSEW)	Apparent lignin			Glucan[2] (% of WSEW)	Total of: pentosan lignin glucan (% of WSEW)
		Klason lignin (% of WSEW)	Acid-soluble lignin (% of WSEW)	Total lignin (% of WSEW)		
10	1.59	28.6	1.7	30.4	60.1	92.1
20	0.97	29.3	1.6	30.9	59.1	90.9
40	0.74	28.2	1.8	30.0	56.6	87.3
80	0.49	31.5	2.2	33.6	55.2	89.3

[1] See footnote of Table 8

[2] Modified anthrone method, corrected for pentosan; see footnote 3 of Table 5

TABLE 12. (Series 6[1], Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 240°C)

Recovery From Gun

Time (s)	Yield of OD SEW (% of original wood)	Moisture content of SEW (%, OD basis)
10	104.7[2]	212
20	75.5	274
40	73.0	286

[1] See footnote of Table 8

[2] Erroneously high

TABLE 13. (Series 6[1] continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 240°C)

Analysis of Whole Steam-Exploded Wood[2], Before Extraction With Water

Time (s)	Pentosan (% of SEW)	Apparent lignin: Klason lignin (% of SEW)	Apparent lignin: Acid-soluble lignin (% of SEW)	Apparent lignin: Total lignin (% of SEW)	Glucan[3] (% of SEW)	Total of: pentosan lignin glucan (% of SEW)
10	15.87	22.0	2.4	24.4	57.8	97.9
20	15.10	22.7	2.6	25.2	56.2	96.4
40	12.84	24.0	2.6	26.6	52.7	92.0

[1] See footnote of Table 8

[2] See footnote 2 of Table 9

[3] See footnote 3 of Table 5

TABLE 14. (Series 6[1] continued, Open Cyclone, 2-L Gun, Acid-Impregnated Wood, 240°C)

Yield of Water Solubles in Water Extract, and Analysis of Water-Washed Steam-Exploded Wood (WSEW) (i.e. Water Insolubles)

Time (s)	Yield of water-solubles[2] (% of original wood)	Analysis of WSEW				Glucan[3] (% of WSEW)
		Pentosan (% of WSEW)	Apparent lignin			
			Klason lignin (% of WSEW)	Acid soluble (% of WSEW)	Total (% of WSEW)	
10	26.8	0.47	32.0	1.9	33.9	56.7
20	26.1	0.45	33.6	2.0	35.6	51.8
40	27.5	0.33	36.8	1.7	38.5	-

[1] See footnote of Table 8

[2] See footnote 2 of Table 10

[3] See footnote 3 of Table 5

TABLE 15. Composition of Permanent Gas in Steam Gun after 1 Minute at 240°C

Gas	Percent of Mixture
Carbon dioxide	91.7
Carbon monoxide	1.1
methane	0.01
ethylene	undetected
nitrogen	5.9 (from original air)
oxygen	0.7 (from original air)
hydrogen	0.6 (uncertain, because of base line)

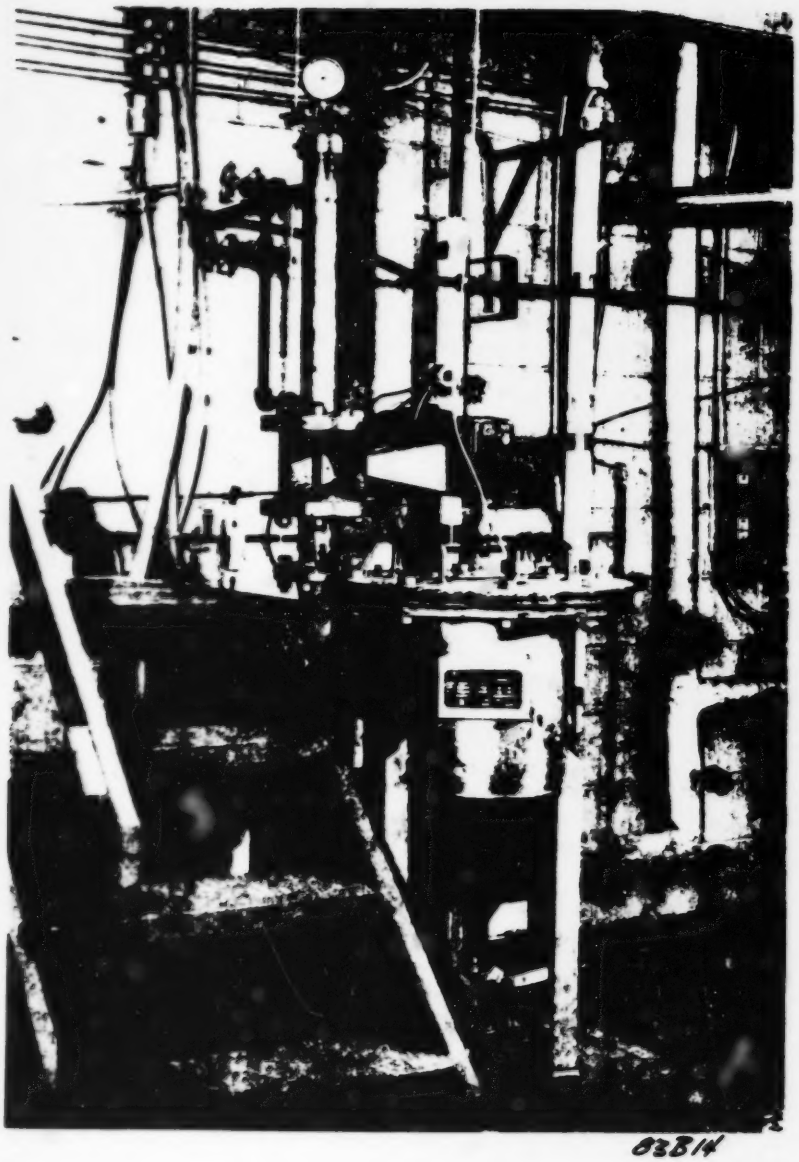

Fig. 1 Two-litre steam gun, before addition of insulation

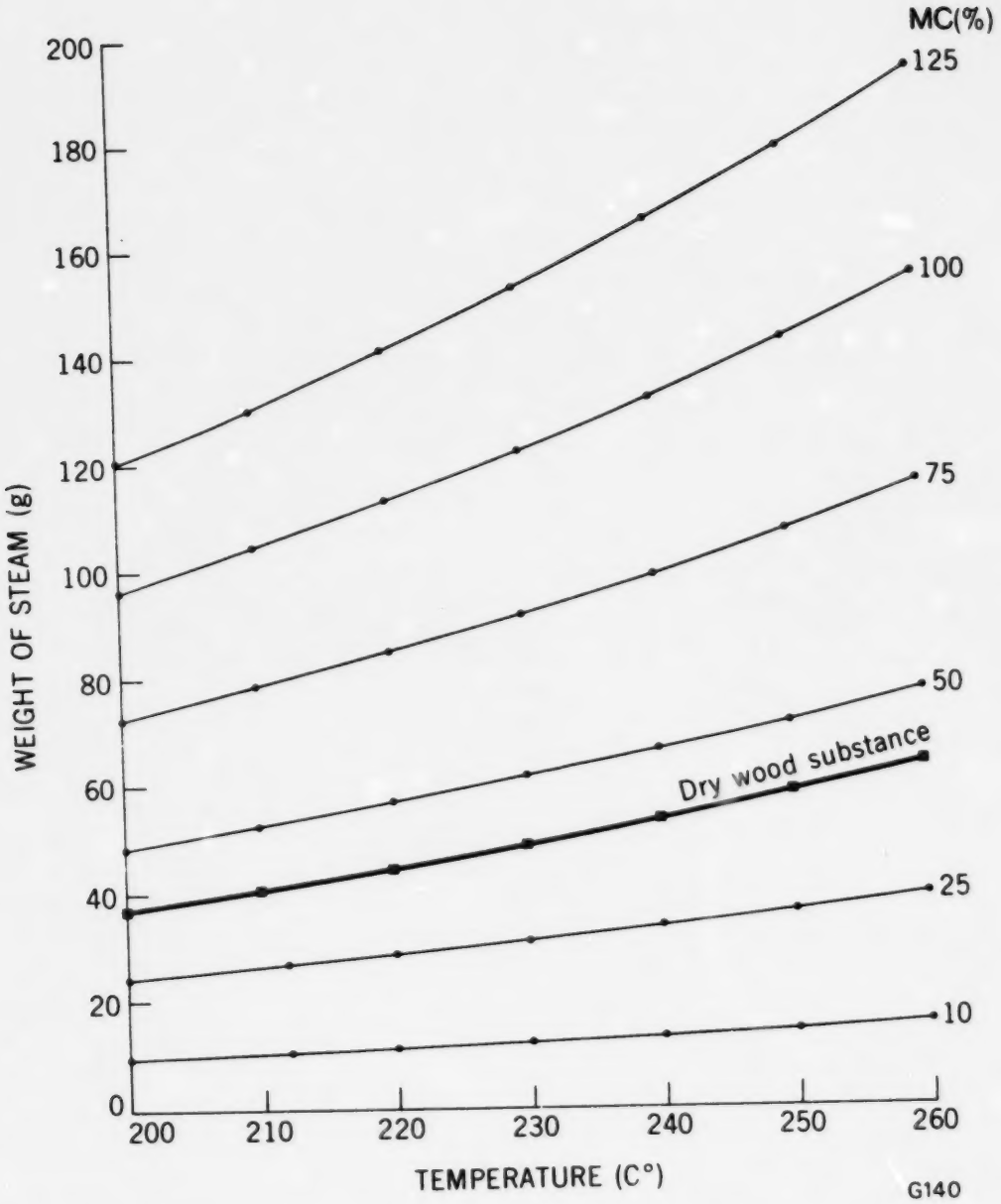

Fig. 2 Comparison of theoretical weight of saturated steam required to heat 250 g of equivalent dry weight of aspenwood from 25°C to steam temperature, with that required to heat only the moisture content of the wood, for moisture contents from 10 to 125 percent (oven-dry basis)

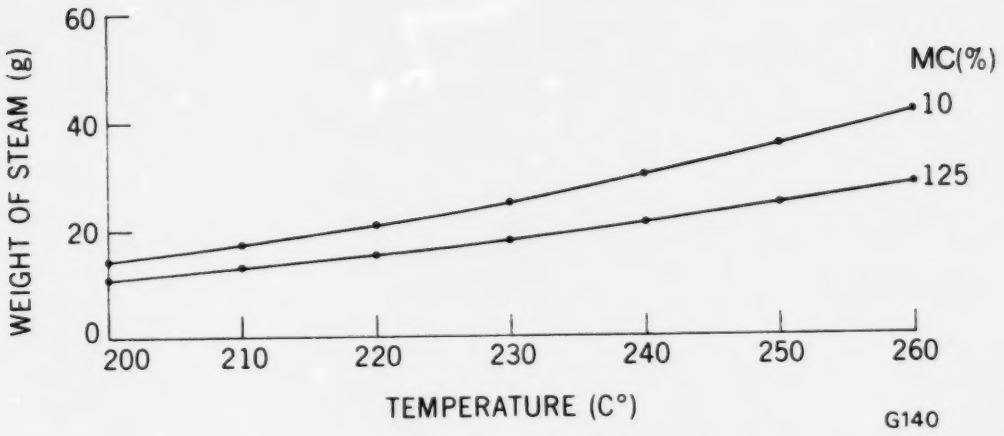

Fig. 3 Comparison of theoretical weight of saturated steam required to fill void volume of two-litre gun charged with 250 g of equivalent dry weight of aspenwood of moisture contents 10 and 125 percent (oven-dry basis)

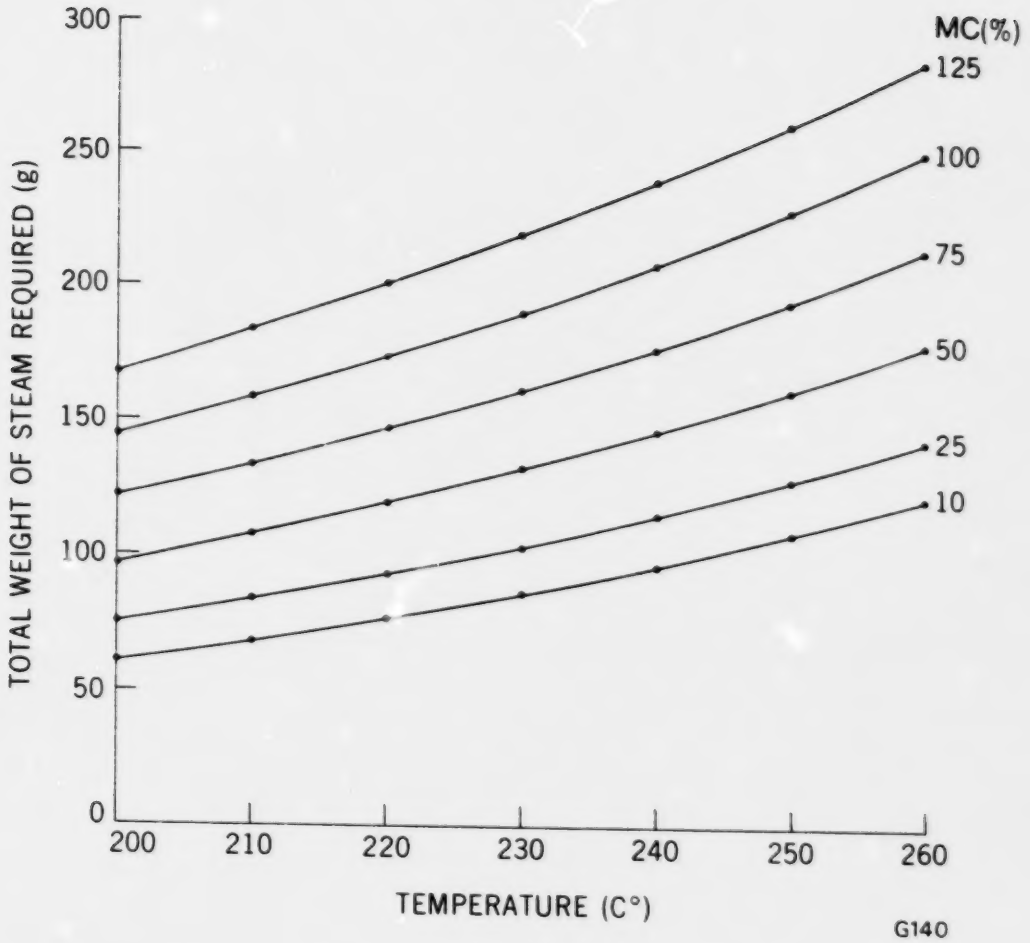

Fig. 4 Comparison of total theoretical weight of saturated steam required to heat 250 g of equivalent dry weight of aspenwood and moisture contents of 10 or 125 percent (oven-dry basis) from 25°C to steam temperature, and to fill void volume

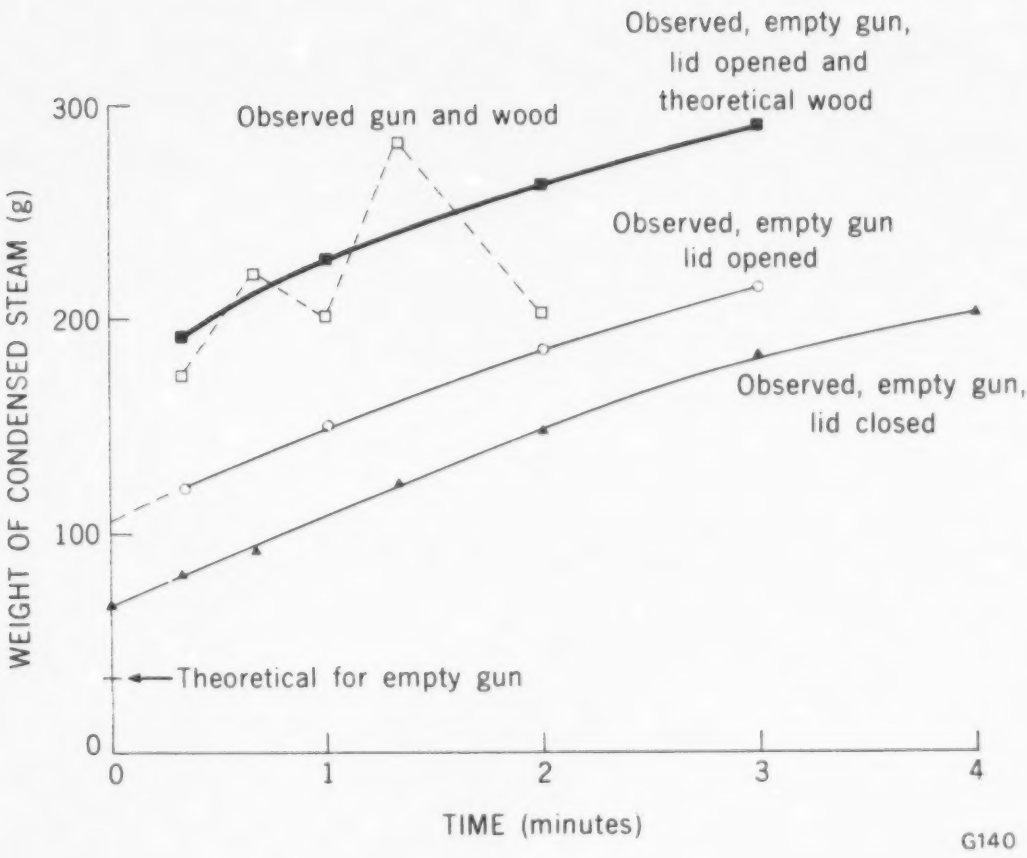

Fig. 5 Comparison of theoretical and observed steam requirements for filling empty two-litre gun, with and without opening of lid, and the additional requirements for heating green aspenwood of equivalent dry weight 100 g and moisture content 51.97 percent (oven-dry basis)

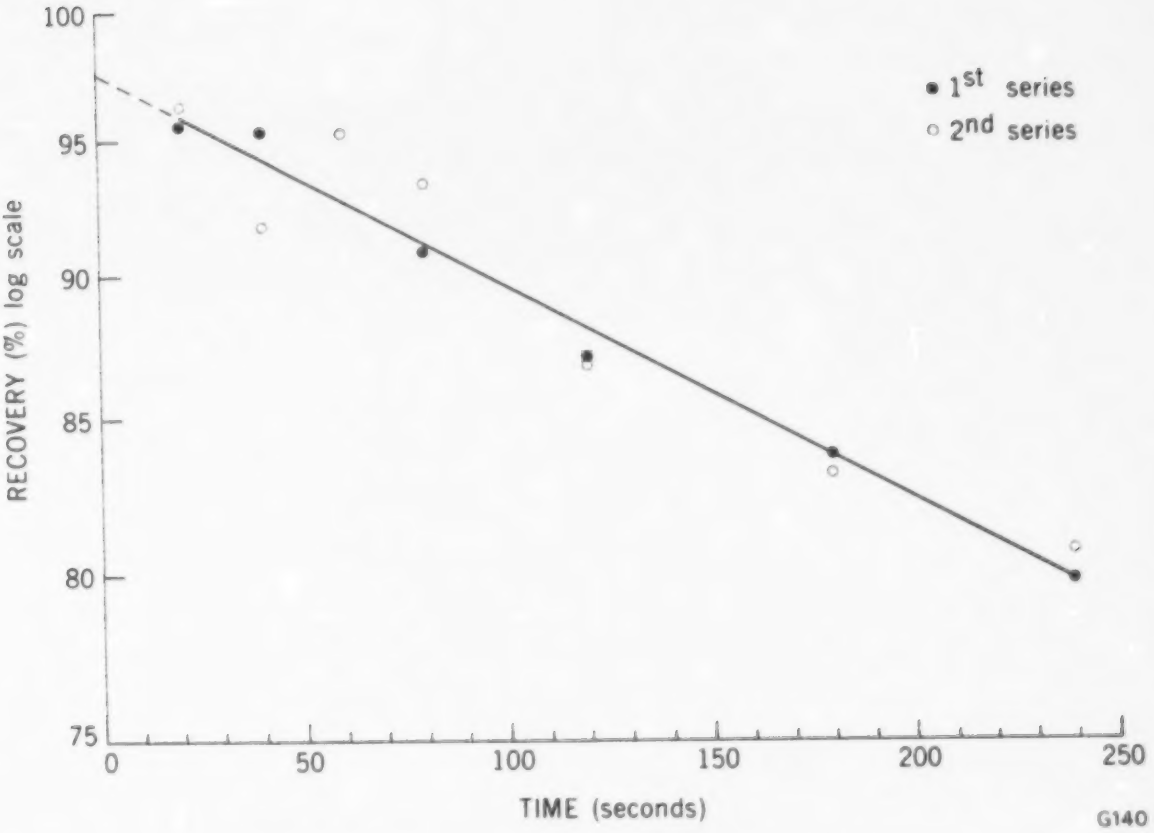

Fig. 6 Recovery of steam exploded wood from two-litre gun, using closed cyclone

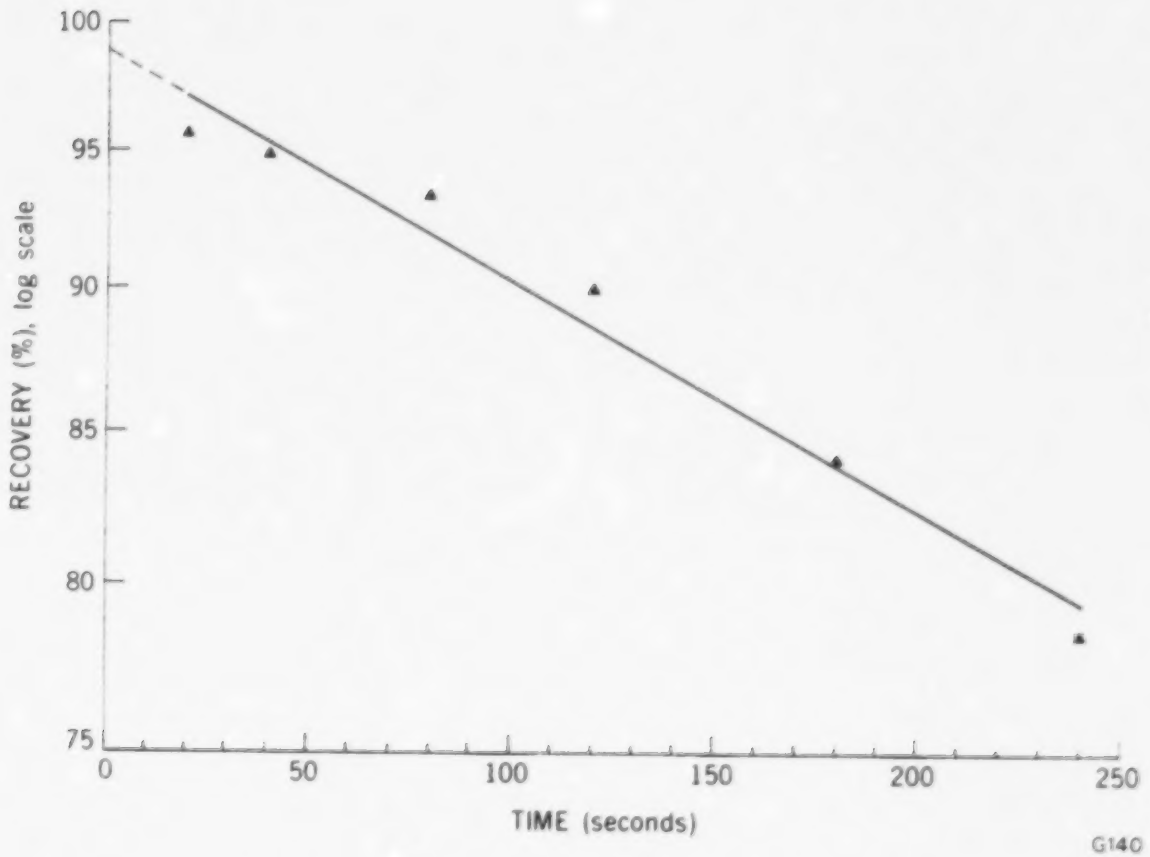

Fig. 7 Recovery of steam-exploded wood from two-litre gun using open cyclone

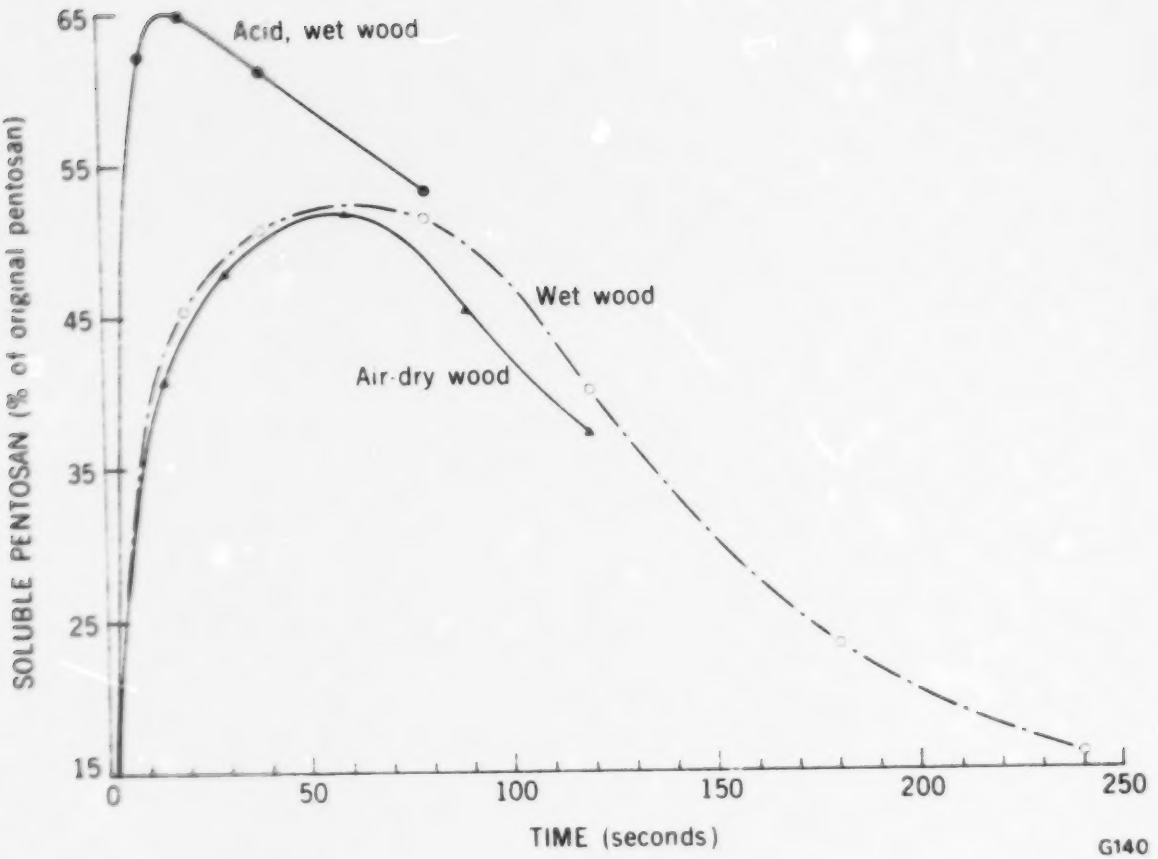

Fig. 8 Comparison of amount of water-soluble pentosan in steam-exploded wood at 240°C, from originally wet or dry, aspenwood, or at 220°C from 0.2 percent sulfuric-acid-impregnated aspenwood

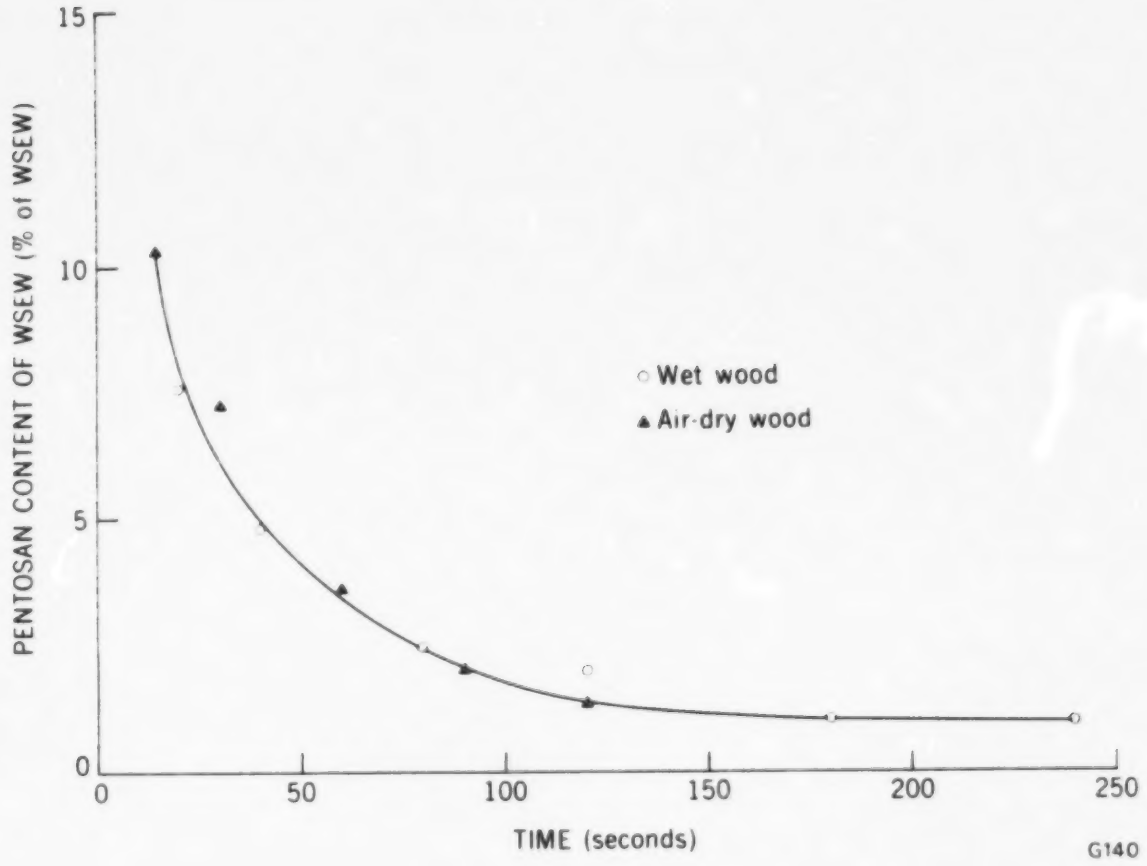

Fig. 9 Comparison of amount of water-insoluble pentosan remaining in water-washed steam-exploded wood (WSEW) from originally wet or dry aspenwood, (pentosan contents from air-dry wood adjusted by multiplication by ratio of pentosan analyses of original woods)

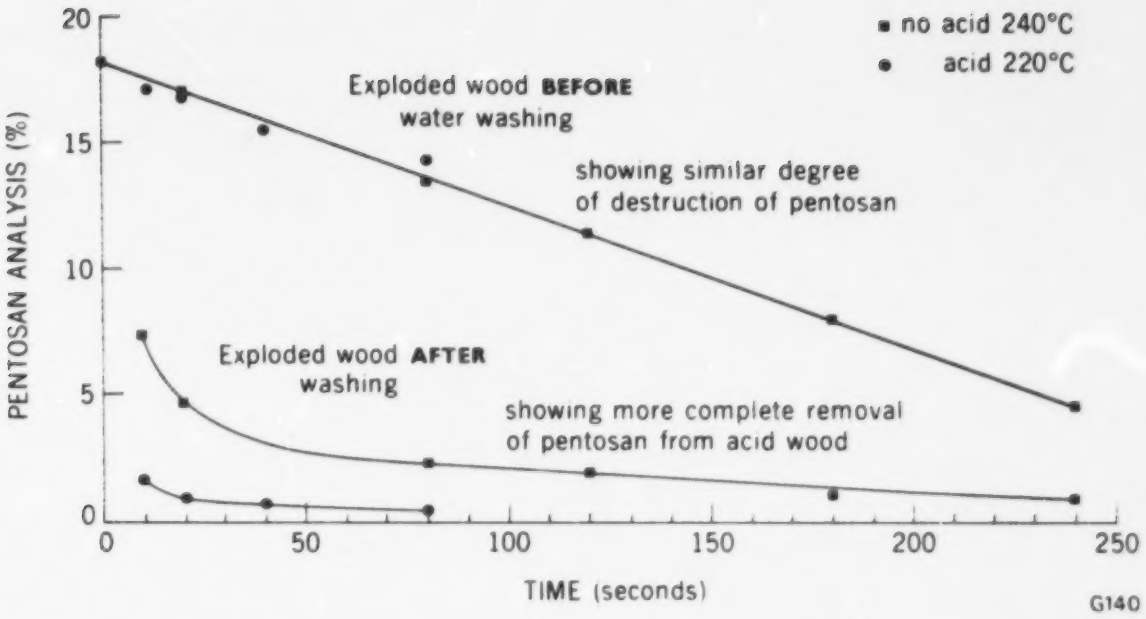

Fig. 10 Pentosan content of steam-exploded aspenwood, before and after water extraction, showing more complete extraction of pentosan from acid-impregnated wood, for similar over-all destruction of pentosan

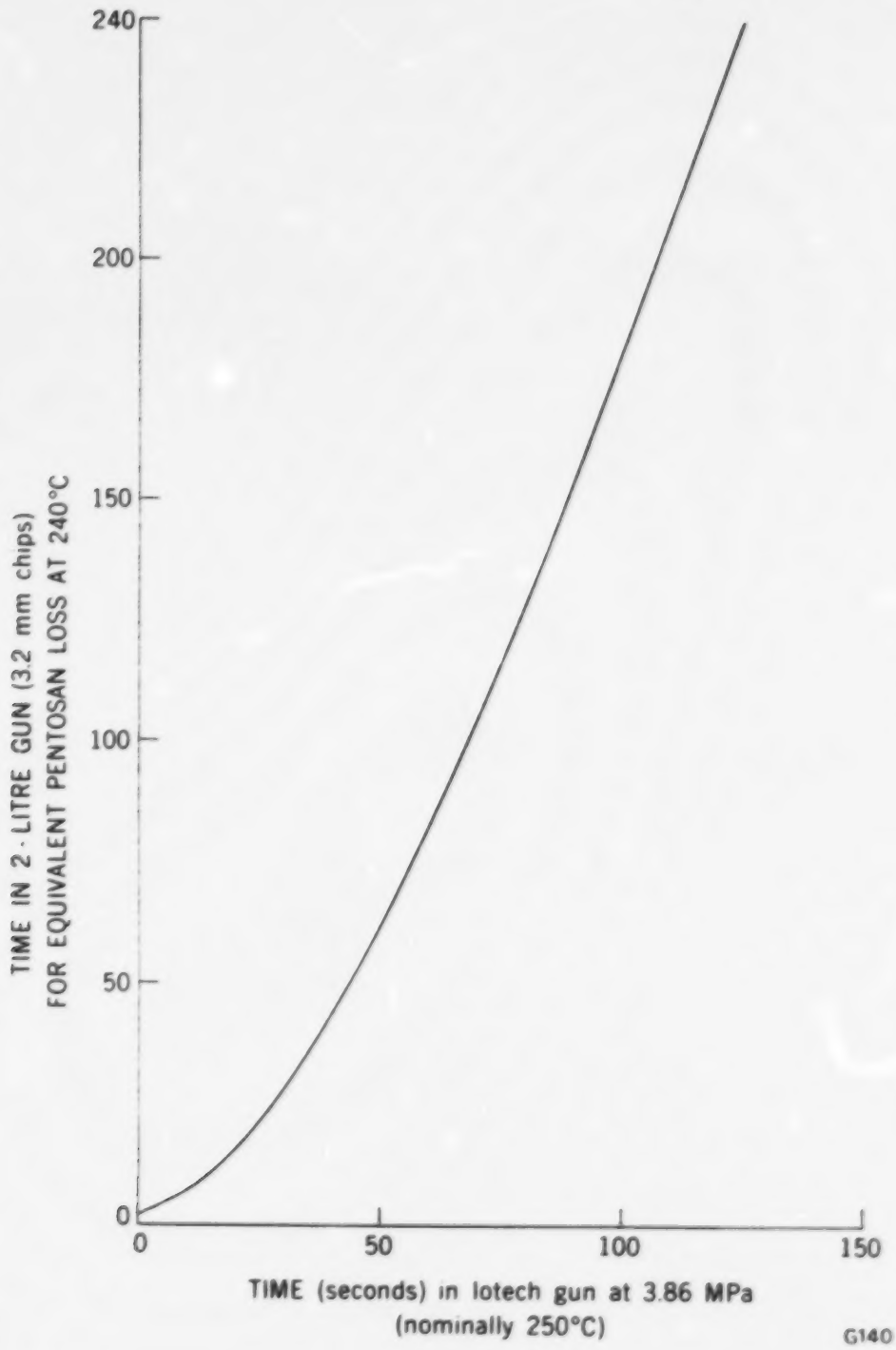

Fig. 11 Comparison of time in Iotech gun at 3.86 MPa (nominally 250°C) to corresponding time in two-litre gun at 240°C for same amount of pentosan destruction

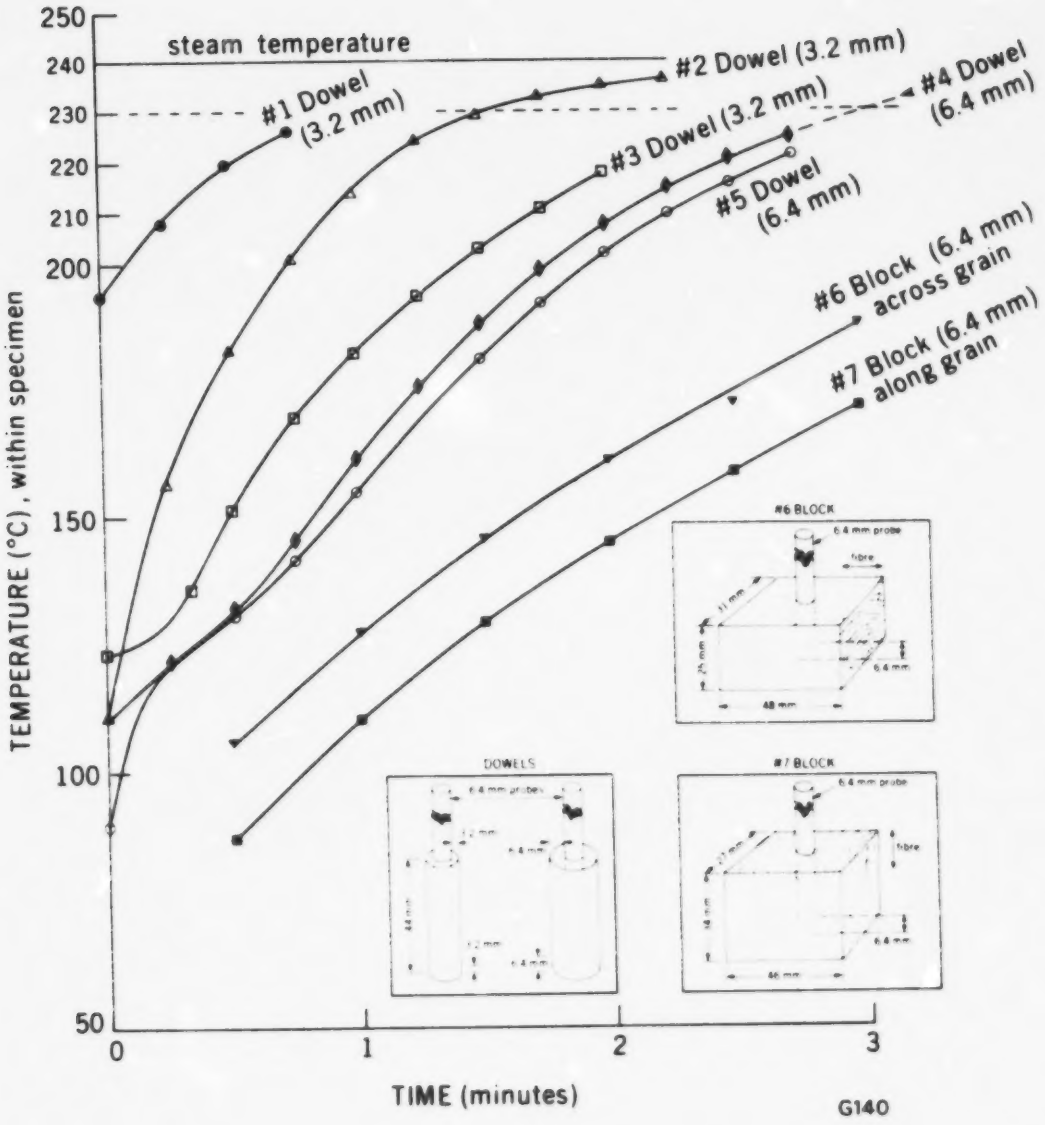

Fig. 12 Temperature rise 3.2 or 6.4 mm beneath surface of green wooden specimens of different shapes and sizes, during heating in steam gun at 240°C

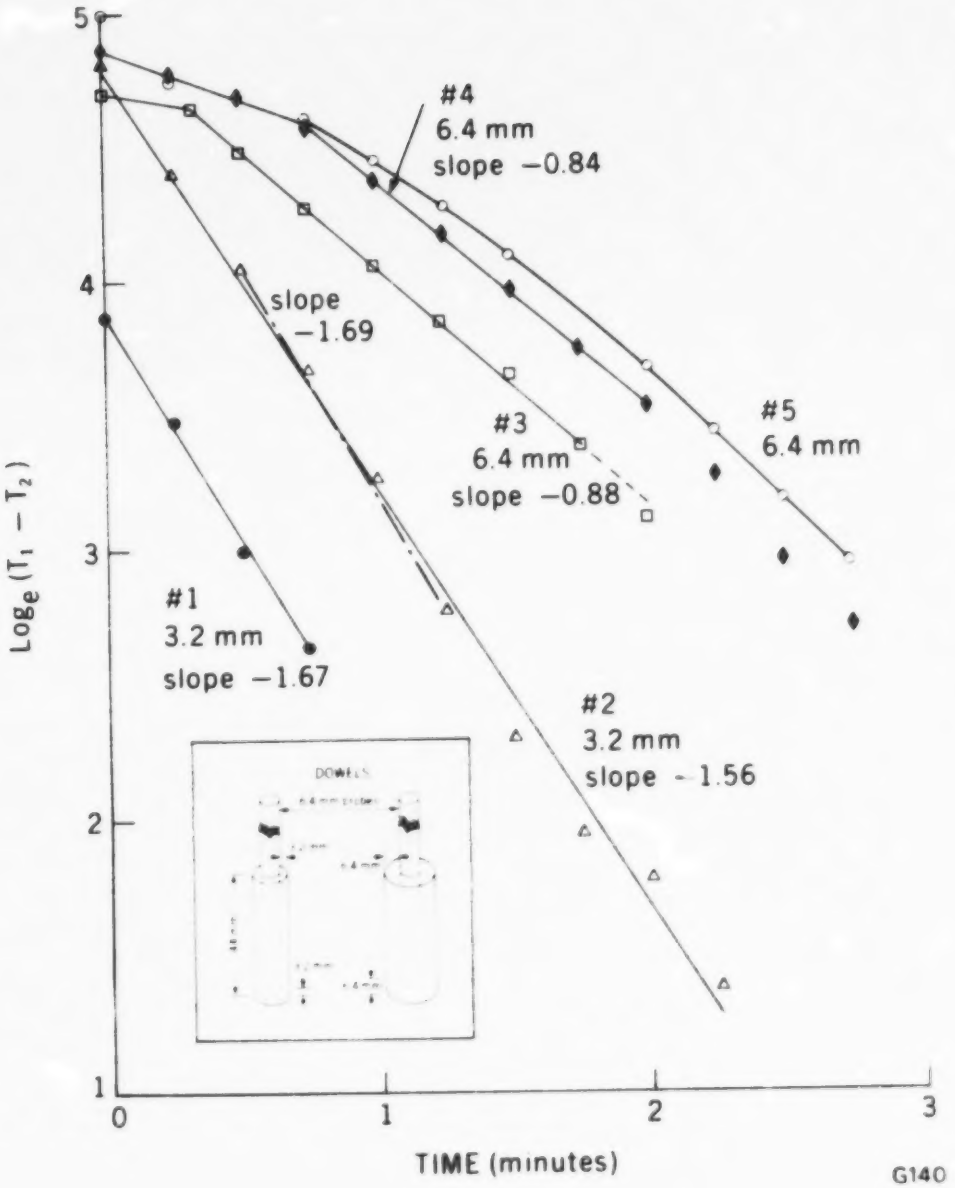

Fig. 13 Decrease in natural logarithm of temperature difference between steam temperature and wood temperature, 3.2 or 6.4 mm beneath surface of dowel-shaped specimens

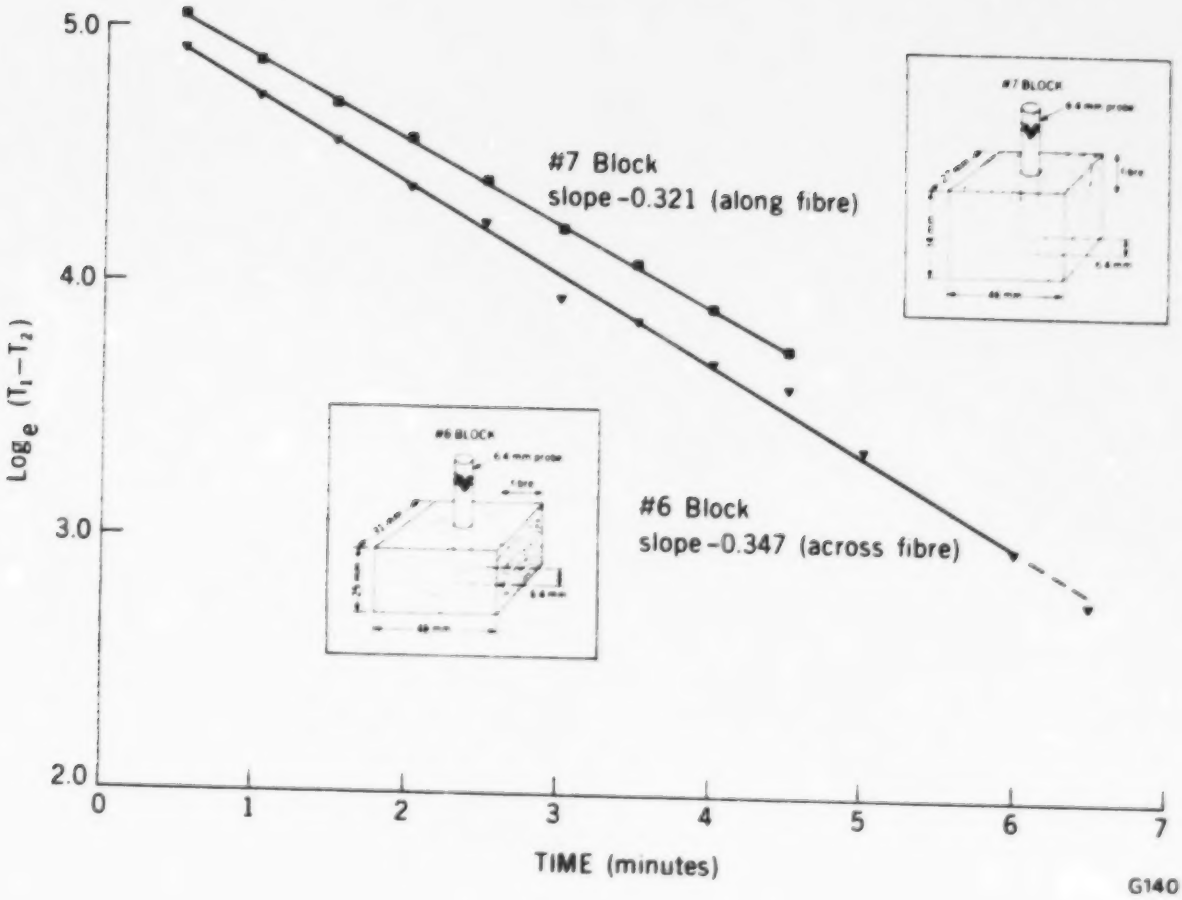

Fig. 14 Decrease in natural logarithm of temperature difference between steam temperature and wood temperature, 6.4 mm beneath surface of rectangular blocks, in fibre direction and across fibre direction

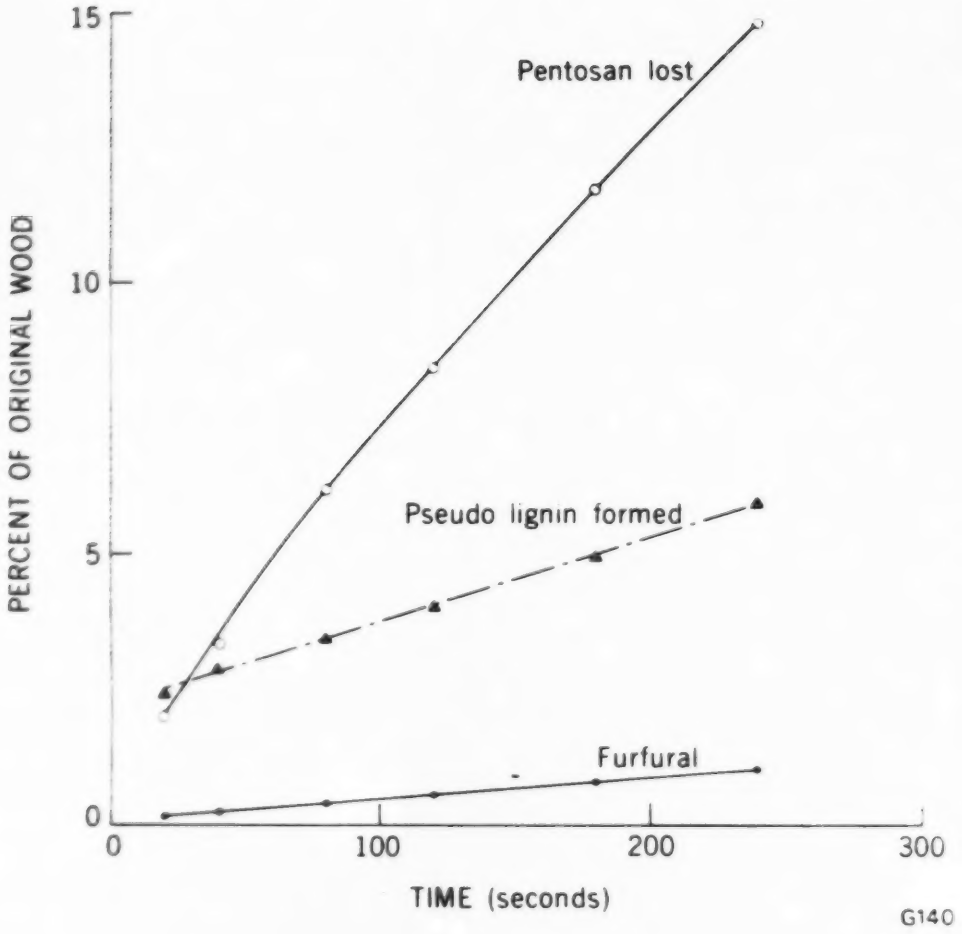

Fig. 15 Destruction of pentosan during steam treatment of aspenwood at 240°C, and accompanying formation of pseudolignin and furfural, showing constant rate of formation of pseudolignin, independant of increasing furfural content

CHAPTER 2

TABLE OF CONTENTS

INTRODUCTION	79
MATERIALS & METHODS	81
Microorganisms	81
Cellulase preparations	81
Assays	81
Cellulase Inhibitor Procedure	82
Substrates for Cellulase Inhibition Studies	82
Fermentation Inhibition Procedure	83
Substrates for Fermentation Inhibitor Studies	83
A. Solvent Extraction	83
B. Selective Extraction Prior to Steam-Explosion	84
C. Selective Extraction Prior to Pressure Treatment	84
D. Distillation	84
RESULTS & DISCUSSION	86
REFERENCES	95

LIST OF TABLES

TABLE 1. Reducing sugars and glucose released by the hydrolysis of various steam-exploded lignocellulosic substrates by T. harzianum E58 cellulases 97

TABLE 2. HPLC analysis of sugars released by enzymatic hydrolysis of steam exploded aspenwood 98

TABLE 3. Reducing sugars and glucose released when the enzymatic hydrolysis of 5% solka floc is supplemented with increasing concentrations of xylose and xylan 99

TABLE 4. Effect of various fractions of steam-exploded aspenwood on fermentation of 5% glucose by S. cerevisiae and Z. mobilis 100

TABLE 5. Possible inhibitors 101

TABLE 6. Effect of various fractions of inhibitor-containing extract on fermentation of 5% glucose by Zymomonas mobilis 102

TABLE 7. Location of inhibitors after ethyl acetate extraction of aqueous extract from exploded aspenwood 103

LIST OF FIGURES

Fig. 1 The effect that added water extracts from steam exploded aspenwood have on the reducing sugars and glucose released after the 24 hr hydrolysis of 5% solka floc by T. harzianum E58 cellulases 104

Fig. 2 The effect that added furfural and hydroxymethylfurfural (HMF) have on reducing sugars released after the 24 hr hydrolysis of 5% solka floc by T. harzianum E58 cellulases 105

Fig. 3 Effect of various concentrations of xylose and xylan on fermentation of 5% glucose to ethanol by Zymomonas mobilis 106

Fig. 4 Ethanol production by S. cerevisiae and Z. mobilis grown for 3 days on 5% glucose supplemented with increasing concentrations of furfural and hydroxymethyl furfural (HMF) 107

INHIBITOR STUDY

INTRODUCTION

Before most native lignocellulosic substrates can be enzymatically hydrolyzed to fermentable sugars there must first be direct physical contact between the cellulosic microfibrils and the enzyme complex. Therefore the substrates must first be pretreated to increase the accessibility of the substrate to the cellulase enzymes. One method which we and other workers (Jurasek, 1979; Taylor, 1980; Saddler et al, 1982) have been using is steam explosion. Steam explosion essentially includes both physical and chemical pretreatments, and reduces the degree of polymerization of the hemicellulose and lignin. A variety of temperatures and retention times have been advocated by other workers using steam explosion (Su et al, 1980; Andren et al, 1975; Puls and Dietrichs, 1980).

Despite the wide range of conditions tested by various researchers, inhibitors were produced which restricted either the enzymatic hydrolysis of the pretreated substrate or the fermentability of the derived sugars (Chahal et al, 1982; Mes-Hartree et al, 1983).

In this study we investigated the nature of these inhibitors using a number of approaches. The inhibition of enzymatic hydrolysis by various lignocellulosic substrates was studied as well as the inhibition of sugar fermentation to ethanol by Zymomonas mobilis and Saccharomyces cerevisiae. To try to better define these inhibitors we tried to isolate them by volatization, steam distillation and solvent extraction. The

above methods were also used to ascertain if a simple and inexpensive treatment could be used to remove or denature these inhibitory materials.

MATERIALS & METHODS

Microorganisms: All the fungi were taken from the Forintek Culture Collection. A spore inoculum was used to initiate growth in shake flasks containing Vogel's medium (Montenecourt and Eveleigh 1977) and solka floc B.W. 300 (Brown & Co., N.W., U.S.A.) as the cellulose substrate. Saccharomyces cerevisiae C495 (NRCC 202001) was grown under standard conditions (Rainbow, 1970). Zymomonas mobilis Z2 (ATCC 29191) was grown in 60 ml serum vials (Miller and Wolin, 1974) using the medium of Rogers et al (1979).

Cellulase preparations:

The cellulolytic fungi were grown for specified times at 28^{o}C on solka floc. Cultures were then filtered through a Reeve Angel glass fibre filter.

Assays

Total reducing sugars were estimated using the dinitrosalicylic acid assay (Miller, 1959). Glucose was determined colorimetrically by the glucostat enzyme assay (Raabo and Terkildsen, 1960). Individual sugars were assayed by high performance liquid chromatography (HPLC) equipped with a Biorad Aminex HPX-87P Heavy Metal column which used water at a flow rate of 0.6 ml/min as the solvent. The column was maintained at 85^{o}C and the refractive index detector maintained at a constant temperature.

Ethanol was determined by gas-liquid chromatography using a 6 ft x 1/8 in. Chromosorb 101 column with flame ionization detector. The detector was kept at 250°C and the column oven operated at 160°C with the injector port at 170°C. Helium which was saturated with formic acid (Ackman, 1972) was used as the carrier gas at a flow of 30 ml/min.

Cellulase Inhibitor Procedure

One gram of the cellulosic substrate was added to 60 ml serum vials. The inhibitory material under investigation was added, and the total aqueous volume made up to 5 ml with distilled water. The pH of the inhibitory extract was adjusted to a pH of 4.8 with sodium hydroxide where necessary. The vials were then capped and autoclaved at 15 psi for 20 minutes. Fifteen ml of T. harzianum E58 culture filtrate cellulase (pH 4.8) at an activity of 30 IU filter paper per gram dry weight residue was inoculated into the vials. The vials were incubated at 45°C on an orbital shaker (100 rpm) for 24 hr., then assayed for reducing sugars and glucose content.

Substrates for Cellulase Inhibition Studies

Aspenwood chips were steam exploded using a high-pressure gun with a 250 cm^3 capacity (Saddler et al, 1982). The substrates were exposed to saturated steam at 560 psi (250°C) for 20 seconds. Some of the substrates were vacuum impregnated with dilute H_2SO_4, and were soaked for 24h at 22°C, prior to steam explosion. Some of the exploded products were also further treated by extraction with 9 volumes of water for 2 hours at room temperature.

The water extract from exploded aspenwood was added to the mixture on a volume to volume ratio, i.e. 10% v/v was equivalent to 10 ml of the water washings added to 90 ml of the enzymatic reaction.

Solka floc was purchased from Brown and Co. Berlin, N.H., U.S.A. Furfural and hydroxymethyl furfural were purchased from Aldrich Chemicals.

Fermentation Inhibition Procedure

The inhibitory material was diluted to a concentration normally found in a 5% slurry of unextracted steam-exploded wood, and the pH of the system adjusted to 4.8 with sodium hydroxide, or phosphoric acid. Twenty millilitres of this extract was dispensed into 60 ml vials containing 1 g glucose and 0.1 g yeast extract. The vials were gassed with N_2, capped and autoclaved for 5 min at 15 psi. One millilitre of a 3 day old S. cerevisiae or Z. mobilis culture was aseptically added, and the vials incubated at 37^{o}C on an orbital shaker (100 rpm). After 2 and 4 days the vials were assayed for reducing sugars, glucose and ethanol.

Substrates for Fermentation Inhibitor Studies

A. Solvent Extraction

Aspenwood was impregnated with 0.2% H_2SO_4 prior to a 25-second steam treatment at 250^{o}C. A 5% slurry (based on dry weight) of the residue was extracted after ? hrs at room temperature. The aqueous extract (300 ml) was extracted twice with ether (2 x 100 ml) or once with ethyl acetate (150 ml). Following removal of the solvents the extract was made up to 300 ml with distilled water.

B. Selective Extraction Prior to Steam-Explosion

Aspenwood wafers were sequentially extracted with alcohol:benzene (1:2), 95% alcohol and alcoholic KOH. Twenty grams of the unextracted and extracted wafers were steam-exploded 80 sec at 248°C. The residue was washed in 400 ml distilled water, then used in the fermentation studies.

The recovered extractives were diluted with distilled water to produce a concentration present in 1.0 g wood. Twenty ml of the extract was tested using Zymomonas.

C. Selective Extraction Prior to Pressure Treatment

Four grams of aspenwood and 6 ml of distilled water were heated 16 minutes in a 20 ml capacity stainless steel pressure bomb in a bath at 230°C. Some of the wood was extracted with alcohol:benzene (1:2) and 95% alcohol prior to heating. The residue was extracted in 80 ml distilled water, and the extract tested for inhibitors.

Xylan and xylose (4 grams) were heated, under the above conditions, in the presence of 6 ml of 0.9 M glacial acetic acid.

D. Distillation

See Section A for details on the water extract used in these studies.

Atmospheric Distillation

Two hundred millilitres of the aqueous extract was distilled under atmospheric pressure in a 500 ml flask. A total of 80 ml of distillate was collected in three fractions.

Vacuum Distillation

Two hundred ml of the above extract was partially evaporated on a Rinco evaporator with full water aspirator vacuum at 40-45^{o}C.

N2 - Bubbling

The extract (20 ml) was placed in a test-tube at room temperature and bubbled with N_2 for 1 hour.

RESULTS AND DISCUSSION

Since the enzymatic hydrolysis of cellulose to glucose is the "raison d'etre" of this pretreatment the effect of substances present in steam-exploded aspenwood on cellulase activity was examined.

A portion of the aspenwood was vacuum impregnated with dilute sulphuric acid prior to steam explosion. After explosion the wood was selectively extracted with water and dilute alkali and then a 5% concentration of these various lignocellulosic substrates was hydrolyzed with T. harzianum E58 cellulases (Table 1).

The percent conversion of the steam-exploded substrates to reducing sugars was comparable to what was obtained when solka floc was used as the substrate, when the lower cellulose content of the pretreated substrates was taken into consideration. The amount of glucose released from the pretreated substrates however was significantly lower. It was noted that the enzymatic hydrolysis of solka floc resulted in a reducing sugars to glucose ratio of 1.3 while this ratio was as high as 4.0 when some of the pretreated fractions were used as substrates. Previously we had found that soaking various lignocellulosic substrates in dilute acid prior to steam explosion resulted in enhanced digestibility of the substrates. Although the conversion of the lignocellulosic substrate to reducing sugars was increased by prior acid soaking, the ratio of reducing sugars to glucose was significantly higher.

When these fractions were water-washed a reducing sugar to glucose ratio similar to that found in the commercial cellulose was obtained (Table 1). Apparently the inhibitor interferes with hydrolysis of the substrate

to glucose but is removed by water-extraction.

To verify that the inhibitory material was present in the water washings, various amounts of this material were added to a control hydrolysis using 5% solka floc as the substrate (Figure 1). It was found that the addition of as little as 2.5% of the water washings resulted in an increase in the reducing sugars to glucose ratio to 1.9. Both reducing sugar and glucose yields were reduced by increased additions of the water-washings.

The data suggested that the β-glucosidase component of the cellulase complex was the most sensitive to the inhibitory material. Other workers (Sinitsyn et al, 1982) have shown that the activity of β-glucosidase was decreased by adding water washings from steam exploded samples. To try to better define the step at which the inhibition was occurring mixtures of the accumulated reducing sugars liberated in the steam-exploded lignocellulosics were assayed by HPLC (Table 2).

Cellobiose was the major sugar detected in the fractions which had been steam exploded. However, after these fractions were water extracted. glucose was the predominant sugar accumulated, with comparatively little cellobiose detected. The unextracted steam exploded fractions were shown to contain xylose which also influenced the reducing sugars to glucose ratio.

Xylose was produced by the enzymatic hydrolysis of the steam exploded samples since 20% of the substrate consisted of hemicellulose and T. harzianum E58 filtrates also contained high xylanase activity

(Saddler and Brownell, 1982). This was of interest as previously it had been suggested (Marsden et al, 1982) that xylose could inhibit the enzymatic hydrolysis of cellulose. We studied the possible innibitory effect that both xylan and xylose might have on cellulose hydrolysis (Table 3) and found that at the concentrations normally found in the hydrolysis reaction neither of these materials restricted cellulose hydrolysis to reducing sugars although less glucose was detected when the hydrolysis reaction was supplemented with xylan. As the T. harzianum E58 cellulase complex also contained xylanase activity these results had to be interpreted carefully as the reducing sugar to glucose ratio was also influenced by the accumulated xylose derived from the hydrolyzed xylan. However it was apparent that supplementing the medium with xylan resulted in a decrease in the glucose detected which also led to an increase in the reducing sugar to glucose ratio.

Our results indicated that the β-glucosidase component of T. harzianum E58 is being inhibited to a greater extent, however, by some substance, other than xylose or xylan, present in unextracted steam-exploded wood.

Other workers (Sinitsyn et al, 1982) have previously suggested that furan derivatives such as furfural and hydroxymethylfurfural, which are produced as degradation products during steam explosion, could also inhibit the enzymatic hydrolysis of lignocellulosic substrates. We tested this hypothesis by adding increasing amounts of furfural and hydroxymethyl furfural to a standard cellulose hydrolysis reaction (Figure 2). As furfural and hydroxymethyl furfural were normally detected at levels of 0.1-0.5 mg/ml and 0.01-0.05 mg/ml respectively in steam exploded aspenwood, it was apparent that neither of the furan derivatives had any appreciable inhibitory effect on enzymatic hydrolysis of these

pretreated substrates.

The inhibitory materials present in the water extract not only inhibit the cellulase complex, they also inhibit the growth of fermentative organisms such as Saccharomyces cerevisiae, Zymomonas mobilis and Klebsiella pneumoniae (Mes-Hartree et al, 1983, Yu et al, 1982) (Table 4).

Z. mobilis, unlike S. cerevisiae, is unable to utilize xylan for growth. A check was made to ensure xylose or xylan were not acting as inhibitors in the unextracted steam-exploded wood fractions. Zymomonas was grown on 5% glucose supplemented with various concentrations of xylose and xylan (Figure 3). The results indicate these substances were not responsible for inhibition of the fermentative process.

Since Zymomonas as well as other fermentative microorganisms are involved in the conversion of cellulose to liquid fuels it is important that these fermentation-inhibiting substances are removed. Z. mobilis was much more sensitive to the inhibitory materials than yeast and would therefore seem to be a useful microbe for detecting the presence of fermentation inhibitors.

Other researchers (Azhar et al, 1981) suggest that the most likely compounds causing this inhibition are furan derivatives. The effect of various amounts of furfural and hydroxymethylfurfural on glucose fermentation by S. cerevisiae and Z. mobilis was tested (Figure 4).

Furfural at concentrations of 5.0 mg/ml inhibited ethanol production in Z. mobilis by 35%, but had no effect on S. cerevisiae. Hydroxymethylfurfural had a more marked effect causing a 50% inhibition of glucose utilization and ethanol production at 3.0 mg/ml for S. cerevisiae and 4.0 mg/ml for Z. mobilis. Furfural was found to be present in our lignocellulosic

reaction mixture at the level of 0.5 mg/ml whereas HMF was present at 0.05 mg/ml. Both these levels are way below the levels which would cause any inhibitory effects, therefore furan derivatives are probably not implicated in the inhibitory effect produced by steam-exploded wood.

The next stage in the study of these inhibitors was to systematically determine their source. A survey of the literature has indicated that inhibitors could be produced from three components of wood (Table 5).

These components are extractives, lignin and carbohydrates. The extractives are a class of compounds which can be removed from wood with alcohol:benzene (1:2) extractions. They include such compounds as tannins, phenols and fatty acids. This class of compounds was investigated as the possible source of the inhibitory substances.

The extractives were removed prior to steam-explosion by a sequential alcohol:benzene (1:2) and 95% alcohol extraction and tested for their effect on ethanol production in Z. mobilis. A 5% glucose solution was supplemented with the extractive fraction at concentrations normally present in unwashed steam-exploded lignocellulosics. They were found to have no effect on glucose fermentation. The water extract obtained after steam-explosion was analyzed for the presence of inhibitors using the above procedure. This extract proved to be inhibitory. These two facts provided fairly conclusive evidence that the extractives are not involved in the inhibitory effect of steam-exploded wood.

Next, the possibility was examined that fragments of lignin, easily removed by hydrolysis, might be the inhibitors. Aspenwood wafers, after a preliminary extraction with alcohol-benzene to remove the extractives already shown not to be inhibitory, were extracted again at room temperature under nitrogen with alcoholic potassium hydroxide. The resulting yellow solution was discarded and the remaining wafers were thoroughly washed with ethanol and then with water. Subsequent steam explosion of the washed wafers, and water washing of the exploded product, gave an extract which, however, inhibited glucose fermentation by Z. mobilis. Apparently, the easily hydrolyzable portions of the lignin are not precursors of the inhibitory materials.

It proved difficult to investigate the degradative breakdown of isolated components of wood, e.g. xylan, using the steam-explosion gun. However, an attempt was made to approximate the gun conditions. The substrate under study was loaded into a small pressure bomb with water, and acetic acid was added where necessary at concentrations reflecting those present during steaming. The bomb was immersed in an oil bath for 16 min at 230°C, and was then cooled rapidly. Aspenwood pre-extracted with alcohol:benzene (1:2) and 95% alcohol, when heated in this manner, produced the inhibitors as did similarly treated unextracted aspenwood. Both xylose, and xylan isolated from aspenwood, after treatment in the pressure bomb produced inhibitors.

The results reported above appear to indicate that at least one of the inhibitors was produced from the xylan of the wood. Certainly the

xylan is the component which undergoes the greatest chemical change during steam treatment, and only a small part of the xylan destroyed is converted to furfural. The possibility, however, that lignin was the source of the principal inhibitors has not been ruled out.

Initial studies were first carried out to see if the inhibitory substances present in the wood following steam-explosion were volatile in nature, and easily removed or denatured by N_2 bubbling or distillation.

The inhibitory fractions were obtained by water washing steam exploded aspenwood substrates. The inhibitors were only present in this water extract with no inhibitory materials detected in the remaining lignin-cellulose fraction. The extract was either bubbled with N_2, or subjected to a vacuum or atmospheric distillation. The volatility of the inhibitors was assessed by testing the action of various fractions of the distillates, or the N_2-bubbled extract, against glucose fermentation in Z. mobilis.

The extract was bubbled for 1 hour with N_2, a procedure known to remove very volatile substances, and then tested for the presence of inhibitors (Table 6). The low ethanol yields indicated that the inhibitors had not been removed by this procedure.

To confirm these results the extract was vacuum distilled on a Rinco evaporator with full water-aspirator vacuum at 40-45^{o}C. Vacuum distillation is a gentler mode of distillation than atmospheric distillation, and reduces the possibility of decomposition of toxic substances. The distillate was collected in two 50 ml fractions, with 100 ml remaining undistilled in the flask. The first fraction collected evidently did not contain an inhibitor, whereas the second fraction

was slightly inhibitory (Table 6). The residue totally inhibited the fermentation of glucose. These results suggest the inhibitor is a relatively inert substance.

The extract was then atmospherically distilled to observe if the toxic substance might be decomposed under this treatment. The distillate was collected in three fractions, with a 120 ml volume remaining in the flask. These fractions along with the residue were tested using Zymomonas, and were all found to contain the inhibitor as indicated by the low ethanol yields. Based on the results obtained with the vacuum distillation the first two fractions were expected to be inhibitor free.

The conflicting results may be due in part to the low pH of the glucose-distillate systems in the atmospherically distilled samples. Apparently, more acid was carried over during the distillation, dropping the final pH of the systems to 3.6-3.8 compared to a pH of 4.3-4.6 in the vacuum-distilled distillate systems. Z. mobilis grows in the pH range 3.8-7.5, depending on the species. The inhibitory effect noted in the atmospherically distilled fractions, as well as the undistilled extract in the vacuum distillation and N_2-bubbling may be a function of the pH rather than inhibitors.

Inhibition of the Zymomonas by toxic substances present in the steam-exploded wood may in fact be due to a summation of the individual effects of many inhibitors. Distillation of the extract into various fractions may serve to separate these inhibitors such that their overall effect is reduced.

We next tried to extract the inhibitory substance from the aqueous extract using ether, which should remove phenolics, organic and fatty acids, and furfural. Surprisingly, the ether did not extract the inhibitory material.

Ethyl acetate extraction was tried next, as it is known to be a more polar solvent than ether. The aqueous and ethyl acetate fractions were analyzed for inhibitors using the Zymomonas model system.

Ethyl acetate was shown to be capable (Table 7) of extracting the inhibitor from the aqueous extract, supplying the means to purify and identify the compounds responsible. The aqueous fraction contained an inhibitor as well, as evidenced by its low growth at Day 2. However the compound was either present at low concentrations, or was not as inhibitory, as Z. mobilis appeared to acclimatize to normal growth after several days incubation.

The exact nature of the inhibitory substances produced during steam explosion have not yet been elucidated, and we are continuing investigations into this research problem. It is known these inhibitors restrict β-glucosidase activity, and directly inhibit glucose fermentation by Zymomonas. Regardless of the nature of this inhibitory substance it is increasingly apparent that production of ethanol from steam-exploded aspenwood will be dependant on a prior water extraction of these substances before efficient hydrolysis and fermentation can be achieved.

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Table 1. Reducing sugars and glucose released by the hydrolysis of various steam-exploded lignocellulosic substrates[a] by T. harzianum E58 cellulases

Substrates[a] (50 mg/ml)	Cellulose content (%)	Reducing sugars (mg/ml)	Glucose (mg/ml)	Reducing sugar/ glucose ratio
SEA	55.2	10.1	3.2	3.2
SEA-W	68.3	11.8	9.6	1.2
SEWS-	54.9	10.6	4.1	2.6
SEWS-W	60.5	11.0	9.1	1.2
0.2 SEA-	55.2	24.5	6.5	3.8
0.2 SEA-W	65.6	24.9	21.5	1.2
0.4 SEA-	55.3	24.1	6.1	4.0
0.4 SEA-W	65.9	25.7	23.5	1.1
Solka floc	98.4	23.6	18.5	1.3

[a] SEA; steam exploded aspenwood; SEA-W, steam exploded aspenwood which was water extracted; SEWS, steam exploded wheat straw; SEWS-W, steam exploded wheat straw which was water extracted; 0.2 SEA, steam exploded aspen with prior soaking in 0.2% H_2SO_4; 0.2 SEA-W, the previous fraction which was subsequently water extracted; 0.4 SEA, steam exploded aspenwood with prior soaking in 0.4% H_2SO_4; 0.4 SEA-W, the previous fraction which was subsequently water extracted. See Methods & Materials; Cellulase Inhibition for details.

Table 2. HPLC analysis of sugars released by enzymatic hydrolysis of steam exploded aspenwood[a]

Substrate (50 mg/ml)	Length of Hydrolysis (days)	Sugars (mg/ml)		
		Cellobiose	Glucose	Xylose
0.2 SEA	1	7.2	5.8	6.7
	5	11.6	7.4	7.1
0.2 SEA-W	1	3.6	15.6	0.5
	5	3.0	23.5	0.5

[a]Hydrolysis conditions are defined in Materials and Methods

Table 3. Reducing sugars and glucose released when the enzymatic hydrolysis of 5% solka floc is supplemented with increasing concentrations of xylose and xylan

Pentose or pentosan added	Concentration added (mg/ml)	Reducing sugars (mg/ml)	Glucose (mg/ml)	Reducing sugar/ glucose ratio
NONE	-	23.7	17.7	1.33
Xylose	5	22.3	17.0	1.31
	10	22.0	16.7	1.32
	15	22.3	16.2	1.38
Xylan	5	22.6	15.3	1.47
	10	22.1	13.4	1.69
	15	24.7	12.7	1.92

Table 4. Effect of various fractions of steam-exploded aspenwood on fermentation of 5% glucose by S. cerevisiae and Z. mobilis

Aspen Sample 5% Glucose	Ethanol Production (mg/ml)	
	S. cerevisiae	Z. mobilis
NONE	21.9	23.4
SE	20.5	ND
SE-W	18.7	18.6
SE-WA	18.8	19.5

Table 5.

POSSIBLE INHIBITORS

EXTRACTIVES	LIGNIN	CARBOHYDRATE
. Resins	. Vanillin	. Furans
. Phenols	. Guaiacol, Catechol + Phenols	. Levulinic Acid
. Fats, Fatty Acid		. Levoglucosan
. Tannins	. p-Hydroxy Benzoic Acid	. Sugar Acids
		. Formic, Acetic Acids
		. Methanol

Table 6. Effect of various fractions of inhibitor-containing extract on fermentation of 5% glucose by Zymomonas mobilis

FRACTION	REDUCING SUGAR (mg/ml)	ETHANOL (mg/ml)	pH
Distilled(Atmospheric)			
20 mls	40.6	1.2	3.71
20 mls	38.6	1.4	3.82
40 mls	40.3	1.2	3.68
Residue	54.1	1.4	3.66
Distilled (Vacuum)			
50 mls	1.9	15.7	4.34
50 mls	21.6	12.5	4.58
Residue	56.0	1.2	3.54
N_2 Bubbled	46.7	1.1	3.76

Table 7. Location of inhibitors after ethyl acetate extraction of aqueous extract from exploded Aspenwood

Fraction	Ethanol (mg/ml)		% Conversion of Glucose to Ethanol	
	Day 2	Day 4	Day 2	Day 4
Ethyl Acetate	3.5	3.5	5.6	5.6
Water	3.6	19.1	6.1	66.7
Control	16.1	18.1	54.6	62.7

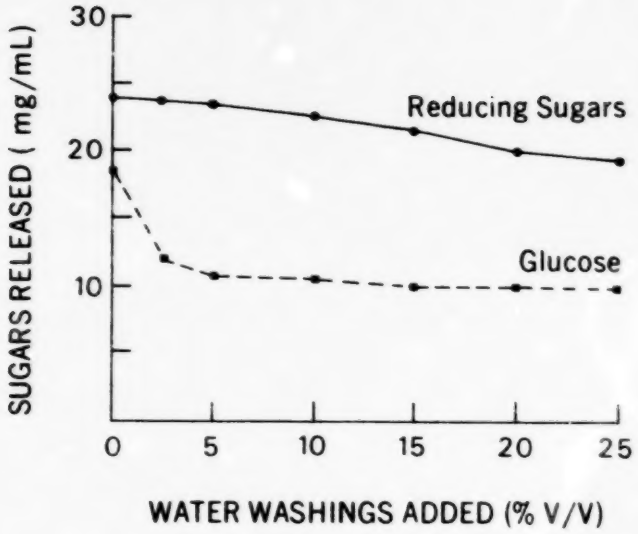

Fig. 1 The effect that added water extracts from steam exploded aspenwood have on the reducing sugars and glucose released after the 24 hr hydrolysis of 5% solka floc by T. harzianum E58 cellulases.

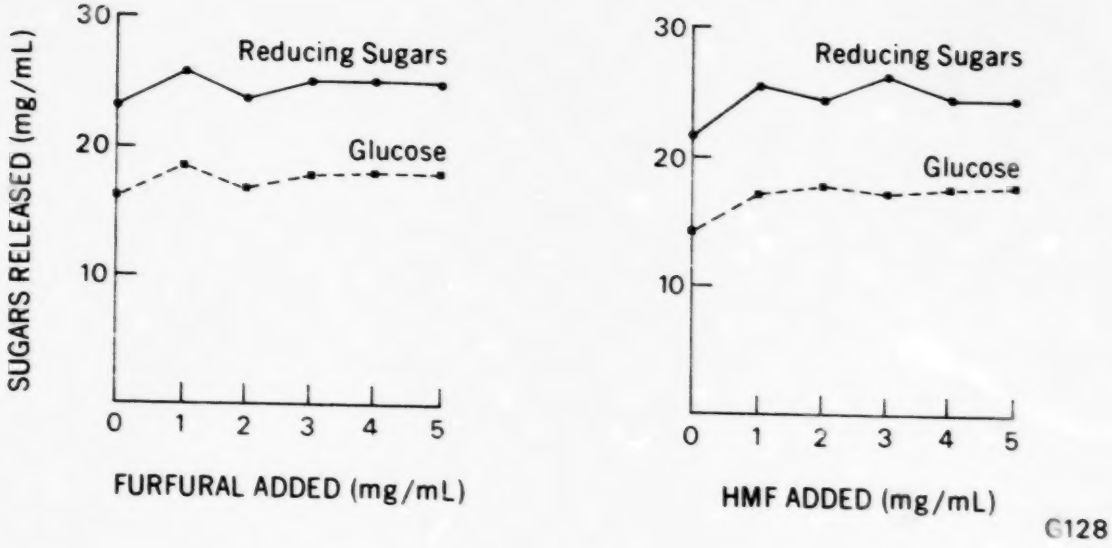

Fig. 2 The effect that added furfural and hydroxymethylfurfural (HMF) have on reducing sugars released after the 24 hr hydrolysis of 5% solka floc by T. harzianum E58 cellulases.

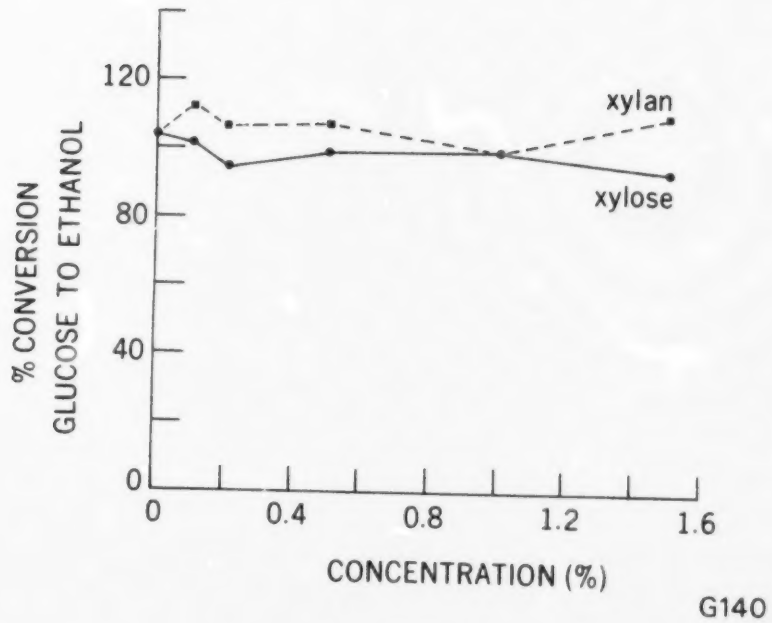

Fig. 3 Effect of various concentrations of xylose and xylan on fermentation of 5% glucose to ethanol by Zymomonas mobilis

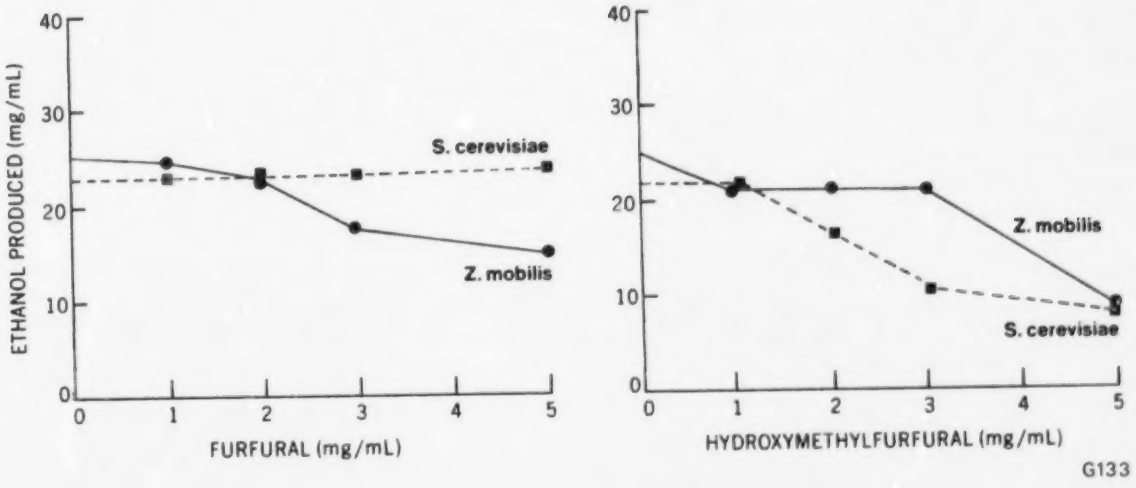

Fig. 4 Ethanol production by S. cerevisiae and Z. mobilis grown for 3 days on 5% glucose supplemented with increasing concentrations of furfural and hydroxymethyl furfural (HMF)

POOR

QUALITY

ORIGINAL